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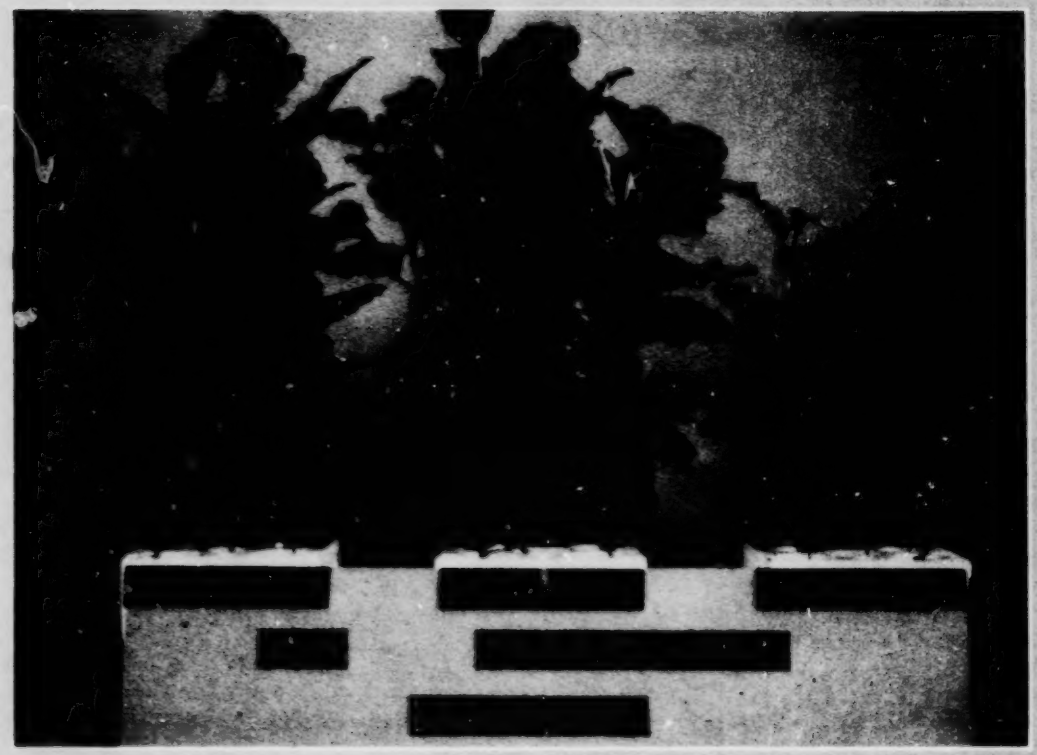
The role of nitrogen fixation in intensive forestry in Canada Part I

Principles, practice, and potential

J.A. Fortin, L. Chatarpaul, and A. Carlisle

Information Report PI-X-28
Petawawa National Forestry Institute



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PETAWAWA NATIONAL FORESTRY INSTITUTE

The Petawawa National Forestry Institute was formed on April 1, 1979, as the result of an amalgamation of the former Petawawa Forest Experiment Station, which had existed for more than 60 years, with the Ottawa-based Forest Management and Forest Fire Research Institutes.

In common with the rest of the Canadian Forestry Service, the Petawawa National Forestry Institute has as its objective the promotion of better management and wiser use of Canada's forest resource to the economic and social benefit of all Canadians. Because it is a national institute, particular emphasis is placed on research into problems that transcend regional boundaries or that require special expertise and expensive equipment that cannot be duplicated in CFS regional establishments. Such research is often performed in close cooperation with staff of the regional centres or provincial forest services.

Research at the Institute is in two main areas:

FIRE RESEARCH AND REMOTE SENSING. Every year in Canada large areas of productive forest are destroyed by fire. Research concentrates on studies of forest fire behaviour, the development of new methods of fire control, the evaluation of fire-fighting equipment and retardants, and the development of computerized fire management systems that are rapidly finding applications with fire-fighting agencies across the country. The environmental and economic impact of forest fires and the use of fire as a silvicultural tool for intensive forest management are also studied.

In remote sensing, investigations are made into the application of modern satellite and airborne remote sensing systems to forestry problems. In this respect, the ARIES digital image analysis system is proving invaluable.

INTENSIVE FOREST MANAGEMENT. As Canada moves into more intensive mangement of its forest to meet expected increases in demand for this important resource, the role of this program will become increasingly important. An extensive reforestation program will require a steady supply of high-quality seed of the desired species. Improved growing stock, obtained through tree breeding and forest genetics research, is highly desirable. Increased emphasis is being placed on using the entire above-ground portion of the tree (biomass), but the effect on the environment of this and other forms of intensive management has to be carefully monitored. Biotechnological methods of improving yield while maintaining site productivity are being investigated.

In support of its research programs, the institute has at its disposal a 98 km^2 area of forest in the northern part of the Petawawa military reserve. Records of experiments and sample plots have been maintained since the 1920s. The forest also serves as a field laboratory for students from local schools, and a visitor centre is operated during the summer months.

Cover:
Testing the efficiency of _Frankia_ isolates to infect, nodulate, and fix nitrogen in _Alnus_ species growing in a N-free medium. Note the poor growth of uninoculated plants in the foreground.

THE ROLE OF NITROGEN FIXATION IN INTENSIVE FORESTRY IN CANADA
Part I

Principles, Practice, and Potential

Information Report PI-X-28

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FOREWORD

ENFOR is the acronym for the Canadian Government's ENergy from the FORest (ENergie de la FORêt) program of research and development aimed at securing the knowledge and technical competence to facilitate, in the medium to long-term, a greatly increased contribution from forest biomass to our nation's primary energy production. This program is part of a much larger federal government initiative to promote the development and use of renewable energy as a means of reducing dependence on petroleum and other non-renewable energy sources.

The Canadian Forestry Service (CFS) administers the ENFOR Biomass Production program component which deals with such forest-oriented subjects as inventory, harvesting technology, silviculture and environmental impacts. (The other component, Biomass Conversion, deals with the technology of converting biomass to energy or fuels, and is administered by the Renewable Energy Branch of the Department of Energy, Mines and Resources). Most Biomass Production projects, although developed by CFS scientists in the light of ENFOR program objectives, are carried out under contract by forestry consultants and research specialists. Contractors are selected in accordance with science procurement tendering procedures of the Department of Supply and Services. For further information on the ENFOR Biomass Production program, contact

ENFOR Secretariat
Canadian Forestry Service
Department of the Environment
Ottawa, Ontario
K1A 1G5

or a CFS research laboratory.

This report is based on ENFOR project P-198 which was carried out under contract (DSS File No. KL011-1-0020) by the Canadian Forestry Service and Laval University.

PREFACE

The purpose of this report synthesize research on the use of nitrogen fixation and mycorrhizae in intensive forestry in Canada, carried out from 1978 to 1983 and supervised by the senior author in the Department of Ecology and Pedology, Faculty of Forestry and Geodesy, Laval University, Ste.-Foy, P.Q. The work was funded by the ENergy from the FORest (ENFOR) program of the Canadian Forestry Service under Projects P-9, P-78, and P-198, and the contracts were negotiated by the Department of Supply and Services.

The work was initiated in response to proposals to intensify use of wood fibre for both energy and conventional forest products. Intense forest use will undoubtedly increase pressures on forest soils, and ENFOR-funded programs of work were initiated at Guelph University, Ontario, and the University of British Columbia to assess impacts of intensive forestry on the soil and to find ways of maintaining soil fertility without excessive use of costly fertilizers. The work was initiated and coordinated by the Petawawa National Forestry Institute, where a core program of cooperative work on environmental impact and biological techniques of soil management (Project PI-11) was also carried out.

Part of this research asked the question, "What is the effect of intensive forestry on soil fertility?" The nitrogen fixation and mycorrhizal research at Laval University attempted to provide a basis for answering the complex but practical question of "How do we maintain soil fertility and increase fibre yield with minimal expenditure of energy and money?"

Although intensive forestry has many effects on the soil, attention was focused on nitrogen because heavy nitrogen losses are likely on intensively harvested and frequently disturbed sites. Available nitrogen is deficient in many forest soils in Canada, and a biological system (nitrogen fixation) to renew nitrogen already exists.

The primary target of the research was the nitrogen-fixing symbioses formed by microorganisms and higher plants. Mycorrhizae were studied because nitrogen-fixing symbioses cannot be studied in isolation from mycorrhizal symbioses. The work on mycorrhizae was confined to fungal symbioses on nitrogen-fixing trees and shrubs, and the participation of mycorrhizae in the fixation process and nitrogen transport. Ideally, nitrogen fixation should be studied in relation to the activities of micoorganisms in the rhizosphere and in the processes of mineralization of soil organic matter, nitrification, and denitrification. This was not feasible at Laval University as the multiple mycorrhizal and nitrogen-fixing symbioses on trees and shrubs are already sufficiently complex to present a major challenge. Nevertheless, parallel work on nitrification and denitrification processes in soils of intensively harvested and managed forests was supported by ENFOR, and carried out at Guelph University and at the Petawawa National Forestry Institute.

The program started in 1978. The ENFOR funds were aimed at providing a scientific basis for future work and providing seed money to attract funds from other organizations. A secondary goal was to establish a centre to train young post-graduates in the field of soil biology in general, and nitrogen fixation and mycorrhizae in particular. If soil management is to succeed, it will be necessary to use little-known forest soil biota, and Canadians with the required skills will be needed.

The report on the program is in two parts. The present volume (Part I) updates and broadens the scope of the contracted literature review, using as a basis the initial 1979 review (Fortin et al. 1979) and two later additions that resulted from ENFOR work (Fortin and Carlisle 1983, Chatarpaul and Carlisle 1983). The review focuses on forestry in Canada and includes new sections on alder taxonomy, soil ecology, nitrogen-fixing processes, and the use of nitrogen fixation in silviculture. Part II describes the main research area and is a synopsis and development of two previous reports (Fortin 1981, 1982) and scientific papers that have been published on the work or presented as masters' or doctoral theses.

Although major research programs are being carried out by Agriculture Canada on nitrogen fixation by legumes, work on actinorhizal non-legumes in forests is being studied at only a few Canadian centres. The main centre for basic work on Frankia, host species such Alnus and Comptonia, and the interaction with mycorrhizae is at the Department of Ecology and Pedology, Faculty of Forestry, Laval University, Ste.-Foy, Quebec (Professors A. Fortin and M. Lalonde). More applied, silvicultural studies are in progress at the Canadian Forestry Service's Petawawa National Forestry Institute, Chalk River, Ontario (Dr. L. Chatarpaul and Mr. D. Burgess). MacMillan Bloedel Ltd. is involved in work on nitrogen fixation of alders in British Columbia, and Ms. J. McLean is studying Shepherdia at the Department of Forest Science, University of Alberta, Edmonton. The Alberta and Quebec Forest Services are involved in the selection of alders, and the Ontario Ministry of Natural Resources is using alders in their poplar programs. Alders are used to reclaim land in the province of Quebec (1.2 million/yr) and in several areas in the western Canada. The National Resarch Council has a directory of nitrogen fixation research in Canada.

Investigations on the use of legumes and non-legumes is in progress in the United States and most of it refers to the forests of the northwest or southeast. Rottink (1980) has compiled a directory of the main projects, and also refers to work overseas.

The authors wish to acknowledge the advice and support of Dr. R. Dobbs, Mr. R.F. Ackerman, and Mr. D.M. Burgess of the Canadian Forestry Service, and the major contributions made by Drs. M. Lalonde, C. Camiré, J.R. Thibault, Y. Laflamme, G. Beaulieu, M. Couture and their students at Laval University.

SUMMARY

The present report attempts to provide forest managers with an information base. It also points out gaps in the knowledge and areas for research.

Intensification of forest management and harvesting will lead to increased pressures on relatively infertile forest soils with possible reductions in levels of nitrogen and organic matter. It will be necessary for foresters to manage the soils, along with trees, if they wish to maintain site productivity. Such maintenance could be through fertilizers but this involves use of fossil fuels, is costly, and can have undesirable side effects on soil microorganism activity and nutrient levels in runoff water. Nitrogen fixation should be considered as a supplementary or alternative soil amendment method.

Free-living nitrogen-fixing bacteria appear to contribute minor amounts of combined nitrogen to forest soils, but it is possible that this contribution has been underestimated. The nitrogen-fixing symbioses of higher plants such as legumes and alders with bacteria such as Rhizobium spp. and Frankia respectively contribute up to 300 kg year^{-1} of nitrogen, more than sufficient to replace potential losses of nitrogen in intensive forestry. The nitrogen-fixing process, however, requires sufficient supplies of elements such as phosphorus, molybdenum, and magnesium, and is sensitive to environmental factors such as light wavelength, pH, water, temperature, and soil oxygen levels. The forester will need to take these factors into account.

There are numerous examples of nitrogen-fixing symbioses improving tree growth and forest soil fertility, and there is evidence in the literature that the use of these symbioses can be economic.

The report discusses the ecology of the nitrogen-fixing organisms in relation to other soil organisms, opportunities for genetic selection, alternative silvicultural techniques, the effects of toxic chemicals, and the taxonomy, improvement, propagation, pathology, and utilization of alders which produce commercial fibre.

It is concluded that there are opportunities to use nitrogen-fixation in forestry in Canada, but there are wide gaps in our knowledge about the ecology and silviculture of plants forming symbioses with Frankia. There is a need to establish well-designed field experiments to examine the interaction of actinorhizal symbioses with the soil and main tree crops, as well as to examine their role as fibre producers.

Résumé

Le présent rapport se veut un essai pour apporter une information de base aux administrateurs forestiers. Il souligne également les lacunes de nos connaissances et suggère des voies pour la recherche.

En intensifiant la gestion et l'exploitation forestières, nous serons amenés à augmenter la pression sur des sols forestiers relativement feu fertiles et à voir le niveau de l'azote et celui de la matière organique s'abaisser. Il importera aux forestiers d'aménager les sols et les arbres s'ils veulent maintenir la productivité du milieu. Pour ce faire, ils pourraient utiliser des engrais mais ces engrais supposent d'un côté l'emploi de combustibles fossiles, sont couteux, et peuvent avois aussi des effets négatifs sur l'activité des micro-organismes du sol et sur la matière nutritive des eaux de ruissellement. La fixation de l'azote pourrait étre une méthode de plus à notre disposition ou une méthode de rechange pour compenser les pestes subies par le sol.

La quantité d'azote fue les bactèries nitriques non en symbioses apportent aux sols forestiers semble minime mais leur apport a été sous-estimé. Les symbioses de fixation d'azote des plantes comme les legumes et les aunes avec les bacteries telles le Rhizobium est et la Frankia fournissent respectivement jusqu'à 300 kg d'azote par an; cela est plus que suffisant pour impenser les pertes éventuelles dans une exploitation forestiere intensive. Le processus de fixation de l'azote nécessite toutefois une apport suffisant d'éléments, tels le phosphore, le molybdéne, le magnésium; il est aussi soumis aux facteurs de l'environnement comme la longueur des ondes lumineuses; le pH, l'eau, la température et la teneur du sol en oxygène. Le forestier devra prendre ces facteurs en considération.

Nombreux sont les exemples de symbioses nitriques qui favorisent la croissance des arbres et la fertilité du sol forestier. Si l'on en juge par les études faites il apparait évident que recourir aux symbioses feut étre économique.

Le présent rapport discute l'écologie des organismes fixateurs de l'azote en relation avec les autres organismes du sol, les opportunités offertes à la sélection génétiques. Il traite aussi de nouvelles techniques de sylviculture, des effets du produits chimiques toxiques. Il touche aussi la taxonomie l'amélioration, la propagation, la pathologie et enfin l'exploitation des aunes qui produisent la fibre commerciale.

En conclusion, on constate que les possibilités d'utiliser la fixation de l'azote dans la foresterie canadienne existent mais fu il y a aussi beaucoup à connaître au sujet de l'ecologie et de la sylviculture des plantes qui vivent en symbiose avec la Frankia. Nécessité donc d'établir un plan bien défini d'essais sur le terrain pour étudier d'un côtë l'influence des symbioses actinorhizales sur la sol et les principaux peuplements, et de l'autre, pour étudier leur rôle en tant que productrices de fibre.

THE ROLE OF NITROGEN FIXATION IN INTENSIVE FORESTRY IN CANADA
Part I

Principles, Practice, and Potential

1.0 INTRODUCTION

One objective of the contracted work was to provide a synopsis of the information in the literature on nitrogen fixation and its potential contribution to forestry in Canada. A preliminary report was issued by Fortin et al. in 1979, and other reports have been written as a result of the Laval/ENFOR work (Fortin and Carlisle 1983, Chatarpaul and Carlisle 1983).

It is hoped that this synthesis would provide forest managers with the information needed to decide whether or not to use nitrogen-fixation to maintain soil fertility and increase fibre yield in combination with, or without, conventional soil amendment methods such as chemical fertilization. The other aim of the report is to identify the main gaps in our knowledge that need to be filled by research, development, or demonstration. There is also additional forestry-oriented material on chemical fertilization, on the ecology of nitrogen fixation, the taxonomy and genetic improvement of alders (Alnus Ehr.), pests and diseases of alder, alder utilization, and economics. The alders are emphasized in the report because these trees, with their potential to modify soils and produce wood fibre, are likely to be the most useful nitrogen fixing systems in Canadian forestry.

The report emphasizes alders but does not attempt to make a case for their use, or other sources of nitrogen fixation, in forestry at this time. It is the forester who must decide, on the basis of available information, which silvicultural technique to use, taking into account management objectives as well as tree species and site characteristics.

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2.0 INTENSIVE FORESTRY AND SOIL NITROGEN

Many Canadian foresters used to operating in the context of harvesting natural forests, long rotations, and minimal crop management are reluctant to accept that soils are likely to degenerate when intensified forest harvest involves the removal of stems, branches, and even twigs and roots. Investigations of the impact of harvesting and management on soil nutrient levels have been usually of the budgetary type, measuring ingress, egress, and net loss of nutrients from one tree harvest to another. Although data from these studies provide a useful information base, the results are inconclusive due to the fact that, although they quantify net losses of nutrients, they do not assess the significance of these losses and their effects on tree growth. Interactive, simulatory computer models have been developed recently that take into account the interaction between soil nitrogen levels and the uptake and needs of tree crops at different stages in their development (Kimmins et al. 1980), and these may eventually provide better management guidance than simplistic nutrient budgets. These models will predict long-term nutrient losses and their effect on tree growth.

The inconclusiveness of the budgetary approach, and the fact that net nutrient losses vary considerably from one tree stand to another, have lulled some forest managers intent upon intensifying harvests into a sense of false security. Nevertheless, the results from the budgetary approaches make it clear that:

i A large part of trees' nutrient content is stored in branches, roots, and leaves, and removal of these components will increase nutrient egress due to harvesting, by 100% or more (Table 2).

ii On rotations greater than 30 years the income of nitrogen in the precipitation may in some circumstances completely replace nitrogen losses incurred by stem harvest, but only partly replace nitrogen egress due to whole-tree harvest (Carlisle 1980, Carlisle and Methven 1979).

iii On very short rotations (less than 20 years) nitrogen in the precipitation cannot always replace nitrogen taken up annually by trees and removed at time of harvest (Chatarpaul and Carlisle 1983).

2.1 Nitrogen removal

Table 1 indicates low levels of nitrogen in the stem wood (0.06% to 0.11%) of four Canadian broadleaved trees greater than 40 years of age, and relatively high levels in branches (0.14% to 0.26%) and leaves (1.05% to 1.49%). Tree bark has a relatively high nitrogen concentration (0.29% to 0.42%). The concentrations of nitrogen in young stem materials are considerably higher than in older materials (Carlisle 1980). Figure 1 is based on hybrid poplar (Populus X) data published by Hansen and Baker (1979). The nitrogen concentration in stem bark declined sharply from 2.03% to 1.00% in the first eight years, and that in stem wood fell from 0.98% in the first year to 0.35% at 2.5 years of age, and then levelled off. There

Table 1: Concentrations of nitrogen in stems, branches, and leaves of broadleaved tree species

Tree species	Stem wood	Stem bark (Nitrogen % O.D.)	Branches	Leaves
Older material more than 40 years of age[1]				
Sugar maple[3]	0.06	0.42	0.16	1.05
Birch[4]	0.07	0.29	0.18	1.17
Aspen[5]	0.07	0.33	0.14	1.49
Oak[6]	0.11	0.37	0.26	1.42
Young material less than 10 years of age[2]				
Hybrid poplar[7]	0.29 – 0.98	1.00 – 2.00	Bark 1.15 – 1.96 Wood 0.49 – 0.94	3.00
Sycamore[8]	0.39 – 0.48	0.77 – 0.98	0.39 – 0.64	1.90 – 2.58

[1] Carlisle (1980). Refers to Canadian material.
[2] Hansen and Baker (1979). Refers to USA material.
[3] *Acer saccharinum* Marsh.
[4] *Betula papyrifera* Marsh.
[5] *Populus tremuloides* Michx.
[6] *Quercus rubra* L.
[7] *Populus* X.
[8] *Platanus occidentalis* L.

Table 2: Nitrogen content of different tree components (after Carlisle 1980)

Species	Age (yrs)	Stems (+ bark)	Branches (+ bark)	Roots	Leaves	Total
	kg ha $^{-1}$ Nitrogen					
Douglas fir[1]	36	125	61	32	102	320
Red pine[2]	39	155	60	75	131	421
Ponderosa pine[3]	50	194	95	37	109	435
Jack pine[4]	40	118	76	37	65	296
White spruce[5]	40	102	127	67	153	449
Hybrid poplar[6]	40	199	82	89	87	457
Red alder[7]	34	309	23	176	100	608

[1] *Pseudotsuga menziesii* (Mirb.) Franco. (Cole *et al.* 1967).
[2] *Pinus resinosa* Ait. (Alban *et al.* 1978).
[3] *Pinus ponderosa* Laws. (Welch and Klemmedson 1975).
[4] *Pinus banksiana* Lamb. (Alban *et al.* 1978).
[5] *Picea glauca* (Moench) Voss. (Alban *et al.* 1978).
[6] *Populus* X (Alban *et al.* 1978).
[7] *Alnus rubra* Bong. (Turner and Singer 1976).

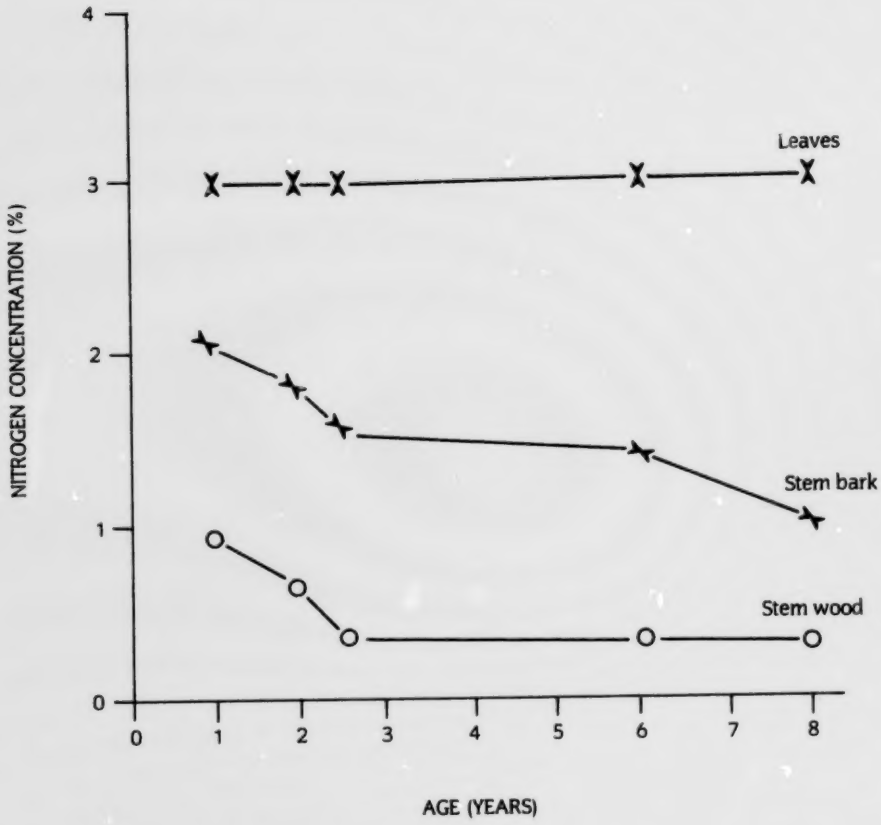

Figure 1: The nitrogen concentration (% O.D.) of stem wood and bark of hybrid poplar (*Populus* X) of different ages. (Based on Hansen and Baker 1979)

was no change in nitrogen concentration in the leaves. These data indicate the need for managers of mini-rotation poplar plantations to avoid cutting too early when nitrogen concentrations in the stem wood and bark are very high. The high biomass production combined with high nutrient levels can result in considerable nitrogen loss.

Although the biomass of branches and leaves is usually low compared with stems, the high nitrogen concentrations (Table 1) result in high absolute nitrogen contents (Table 2) in these components. In Table 2 the white spruce branches (without leaves) account for 127 kg ha^{-1} of nitrogen and the leaves 153 kg ha^{-1}, compared with 102 kg ha^{-1} in the stems. Red alder has relatively little nitrogen in the branches (23 kg ha^{-1}) due to the low branch biomass, but the alder roots (176 kg ha^{-1}) and leaves (100 kg ha^{-1}) contain a great deal of nitrogen.

2.2 Nitrogen uptake

Nutrient uptake by the trees to be harvested is a good index of the continuous needs of the growing trees for nutrients, and how much nutrient will need to be replaced after harvesting. Hansen and Baker (1979) provided nitrogen accumulation rates (equivalent to uptake) for stems and branches of poplar (Populus), sweetgum (Liquidambar styraciflua L.), and sycamore (Platanus occidentalis L.). The uptake of nitrogen in 1 to 20 year-old poplar plantations varied from 6 to 60 kg ha^{-1} $year^{-1}$. Sweetgum plantations accumulated 5 kg ha^{-1} $year^{-1}$ over the first 11 years, while 1 to 4-year-old sycamore stands accumulated 1 to 40 kg ha^{-1} $year^{-1}$ of nitrogen. In some cases nitrogen entering ecosystems in rainfall (about 5 kg ha^{-1} year) is enough to replenish the accumulation in bark and wood of stems and branches. In other cases there is a shortfall of 35 to 81 kg ha^{-1} $year^{-1}$ of nitrogen.

No conclusive published nutrient data were located for short rotation poplars in Canada, but it is possible to make a crude calculation. There are records of very high yields of up to 16 O.D. tonnes ha^{-1} $year^{-1}$ in hybrid poplars grown in small plots (Raitanen 1978, Ek and Dawson 1976), and Zsuffa (1971) recorded a mean annual increment of 18 m^3 ha^{-1} for 18-year-old poplar in Canada, equivalent to an m.a.i. of 7.2 tonnes ha^{-1}. For the purposes of this calculation a conservative yield of 7 000 O.D. kg ha^{-1} $year^{-1}$ of stem material will be used. Poplar stem wood and bark at the age of about 5 years have nitrogen concentrations of about 0.26% and 1.40% respectively (Fig. 1). The overall stem material (at 5 years, stem and branches need not be differentiated) has a nitrogen concentration of about 0.5% O.D., a figure that is supported by data for trembling aspen (Populus tremuloides Michx.) (Henry 1973). Ignoring the leaves, which are deciduous, the uptake of nitrogen in the stem material is about 35 kg ha^{-1} $year^{-1}$. This ignores root uptake but, conservatively, roots will take up about another 12 kg ha^{-1} of nitrogen per year, totalling 47 kg ha^{-1} year. The stand will need at least this quantity of nitrogen each year to maintain growth. Allowing for nitrogen income in the rainfall and from other natural sources, the nitrogen deficit will be at least 40 kg ha^{-1} $year^{-1}$.

Tree stands take up different quantities of nutrients and accumulate them at different rates during their life. In the case of loblolly pine

(Pinus taeda L.) in the USA nitrogen uptake was highest (8.3 kg ha^{-1} $year^{-1}$) in the first 10 years, declining to 3.0 kg ha^{-1} $year^{-1}$ at 40 years (Table 3) (Alban 1979, Switzer et al. 1968). It is of interest to note that phosphorus and potassium uptake peaked later at 10 to 20 years, and calcium at 20 to 30 years. Fertilization and other soil management techniques should be carried out with these variations in mind.

2.3 Nitrogen income

Nitrogen and other elements enter forest soils naturally in precipitation, aerosols, mineral weathering, and microbial fixation of nitrogen gas.

2.3.1 Precipitation

On average, nitrogen enters in the rainfall at a rate of 5.0 kg ha^{-1} $year^{-1}$ (Carlisle 1979). Data for Canada are scarce due to many analyses being made on an event basis rather than a daily basis, but the establishment of new meteorological networks will make better data available in the near future. Current data for Lake Superior, Lake Huron, and Nova Scotia indicate that nitrogen reaches the soil in rain and snow at rates of 6.8, 8.7, and 3.9 kg ha^{-1} $year^{-1}$ respectively, and that the income is very variable in Ontario (3.0 to 41.4 kg ha^{-1} $year^{-1}$) due to local zones of high pollution by industry (Carlisle 1980).

The income of nutrients in aerosols (minute droplets that enter forest ecosystems even during rain-free periods) has not been studied a great deal and little data are available. Nevertheless, it is known that in Germany and Scotland (Table 4) the income of bases in aerosols is sometimes of the same order as the income in the rainfall and cannot be ignored. Information about the nitrogen content of aerosols is needed, but the difficulties of measurement of aerosols impacting on forest canopies are formidable. A high proportion of bases in aerosols are of oceanic origin, sea water being rich in bases (particularly magnesium) and low in phosphorus (Table 5). Nitrogen in aerosols impacting on forest foliage is likely to come from many sources (organic dust, industry, denitrification, etc.) but no reliable figures have been located. This income is not likely to exceed that in rainfall (about 5 kg ha^{-1} $year^{-1}$).

2.3.2 Mineral weathering

The income of nutrients from weathering of rock and soil minerals is difficult to measure on a kilogram per hectare basis. There are crude data available for the release of bases (10 to 50 kg ha^{-1} $year^{-1}$) from plagioclase, biotite, and feldspar minerals using the weathering rind approach and there have been attempts to use the mass balance method to measure release by weathering, but no reliable measurements of nitrogen income from mineral weathering have been located (Carlisle 1980).

Although there are huge quantities of nitrogen locked up in the sedimentary rocks (0.4×10^9 tonnes) and primary rocks (193×10^9 tonnes) of the planet Earth (Burns and Hardy 1975), tree roots only have access to a small proportion of this mineral nitrogen reservoir. In the short term nitrogen income from parent materials is not likely to be large. Nevertheless, in the long term the nitrogen in the rocks and minerals could

Table 3: Mean annual accumulation of nutrients in *Pinus taeda* biomass on good sites (Alban 1979; Switzer *et al.* 1968)

Nutrient	Age (years)			
	0 to 10	10 to 20	20 to 30	30 to 40
	(Mean kg ha $^{-1}$ year $^{-1}$ accumulated)			
Nitrogen	8.3	8.0	4.9	3.0
Phosphorus	0.8	1.8	0.5	0.1
Potassium	4.1	5.6	3.5	1.7
Calcium	2.6	4.9	5.2	3.8
Magnesium	0.9	1.5	1.7	1.4

Table 4: Income of elements in rainfall and aerosols (after Miller 1979)

	Ca	K	Mg
	kg ha $^{-1}$ year $^{-1}$		
Beech *Fagus sylvatica* L. – Germany[1]			
Rainfall	13	4	3
Aerosols	6	5	1
Corsican pine *Pinus nigra* ssp. *laricio* (Poir) Palibrin Coste – Scotland[2]			
Rainfall	3	3	3
Aerosols	4	7	2

[1] Mayer and Ulrich 1974.
[2] Miller, Cooper, and Miller 1976.

Table 5: Ratios of nutrient element content to sodium content in sea water (after Kalle 1943)

Ratio	Sea water
K/Na	0.036
Ca/Na	0.039
Mg/Na	0.120
P/Na	0.000005

play a significant role. Nitrogen is held in the fundamental rocks as nitrides (NO_2^-) of iron, titanium, and other metals (Wollum and Davey 1975), and also occurs in the ammonium (NH_4^+) form attached to silicate minerals, for example in the C horizons of soils (Adams and Stevenson 1964). These nitrogen-containing minerals release their nitrogen very slowly, but should not be ignored.

2.3.3 Microbial fixation of nitrogen gas (N_2)

The quantities of nitrogen fixed by different organisms and symbioses will be discussed in other parts of the report. At this point it suffices to refer to Table 6 and point out that many of the symbioses in legumes and non-legumes fix more than 85 kg ha^{-1} $year^{-1}$ of nitrogen, and some fix as much as 300 kg ha^{-1} in favourable situations. Some estimates of nitrogen fixation rates in forests may be low, as the amount of nitrogen fixed in tree root rhizospheres is difficult to quantify on an area basis under natural conditions.

The nitrogen-fixing symbioses have the potential to more than offset shortfalls in nitrogen associated with intensive harvesting, provided the plants can compete successfully and grow in forest situations. Their nitrogen fixation rates far exceed the rates of income in precipitation.

2.4 Nitrogen balance

The previous data indicate that in some intensive forestry systems, particularly where short rotations are involved, annual nitrogen uptake in the stem and branches will exceed the income in precipitation, aerosols, and mineral weathering. In most forest soils in Canada, fixation of nitrogen by free-living soil bacteria will make only a minor contribution, although the total income of nitrogen from asymbiotic fixation by bacteria may prove to be of more importance once fixation in other non-soil niches in forest ecosystem is understood better. Different estimates of potential nitrogen deficits associated with intensive silviculture described in this report indicate that about 30 to 80 kg ha^{-1} $year^{-1}$ of nitrogen will need to be replaced to maintain growth and site productivity in some situations. The nitrogen-fixing symbioses of temperate rhizobial legumes and actinorhizal non-legumes have the potential to fix up to 300 kg ha^{-1} $year^{-1}$, which is more than enough to make good the potential nitrogen deficit. Whether or not legumes and actinorhizal non-legumes can be used in forest management will, of course, depend upon their compatibility with the forest sites and the main tree crops.

Nitrogen loss will not be the only soil problem encountered when fast-growing tree species, short rotations, and harvesting of stems and other tree components are used. There may well be losses of phosphorus and bases that will have to be replaced by fertilization, but a more serious potential loss is forest soil organic matter. This organic matter provides the substrate for forest soil microorganisms involved in nitrogen transformations and provides ion-exchange sites that retain otherwise mobile ions in the soil system; adequate levels are essential for soil fertility.

Table 6: Rates of nitrogen fixation

	Nitrogen fixed (kg ha $^{-1}$ year $^{-1}$	Reference
Free-living bacteria		
Clostridium pasteurianum	30.0	Postgate 1978
Legume symbioses		
(*Rhizobium* symbiont)		
Glycine max (soybean)	57 – 94	Burns & Hardy 1975
Pisum spp. (garden pea)	85	"
Apios americana (ground nuts)	47 – 91	"
Lens esculenta (lentil)	85	"
Vicia spp. (vetches)	85	"
Trifolium spp. (clovers)	105 – 160	"
Medicago sativa (alfalfa)	128 – 300	"
Lupinus spp.	150	"
Acacia spp.	270	"
Non-legume symbioses		
(*Frankia* symbiont)		
Alnus rubra Bong	100 – 300	Tarrant & Trappe 1971
A. crispa Ait. Pursh.	62 – 157	"
A. rugosa (Du Roi) Spreng	79 – 150	"
A. incana L. (Moench)	50	"
A. glutinosa L. (Gaertn.)	125	Cole *et al.* 1978
Casuarina spp.	50 – 200	Haines & de Bell 1979
Ceanothus spp.	60 – 150	Stewart 1982
Coriaria spp.	90 – 200	Haines & de Bell 1979
Elaeagnus spp.	2 – 15	"
Hippophaë spp.	80 – 197	"
Myrica spp.	52	Sprent and Scott 1979
Forest ecosystems[3]		
Alder (*Alnus* spp.)	12 – 300	Fortin *et al.* 1979
Beech (*Fagus*) forest	0.4	Burns & Hardy 1975
Forest and wood (global)	10	"
Agriculture (global)		
Cultivated Land		
Legumes (general)	140	Burns & Hardy 1975
Rice paddy	35	"
Other	10 – 55	"
Prairie	15	"
Unused land	2	Burns & Hardy 1975
Sea	1	Burns & Hardy 1975
Tropical rain forest	14 – 88	Burns & Hardy 1975
Sea	1	Burns & Hardy 1975

[1] More detailed lists of quantities of nitrogen fixed by symbioses are given in Nutman (1976) and Stewart (1982).

[2] Nitrogen fixed by tropical and sub-tropical plants and microbial symbionts are listed by Nutman (1976). Examples range from 62 kg ha $^{-1}$ year $^{-1}$ for *Lotonosis* to 897 kg ha $^{-1}$ year $^{-1}$ for *Leucaena*.

[3] Recent data for *Alnus rubra* Bong and *Alnus sinuata* (Regel) Rydb. on Vancouver Island, B.C. show that these trees fix 130 kg ha $^{-1}$ year $^{-1}$ and 20 kg ha $^{-1}$ year $^{-1}$ respectively (Binkley 1981).

3.0 MAINTAINING SOIL NITROGEN LEVELS

Traditionally Canadian foresters have managed forest soils by fertilization in conjunction with site preparation by slash-burning, scarification, and weed control by herbicides. Manipulation of the vegetation cover, such as by changing tree species or by using mixtures, can also be used to modify soils. The composition of exudates of living leaves and roots, and characteristics of leaf and root litter under different tree canopies, greatly affects the physical and chemical properties of soil organic matter and activities of the soil biota. Forest vegetation is seldom used intentionally to manage forest soils in North America except in reclamation of sites disturbed by mining.

Nitrogen-fixing organisms and symbioses have not been widely used in Canada in the management of forest soils, although they have considerable potential as soil stabilizers and as sources of nitrogen and organic matter. Legumes are widely used in agriculture, although their role as sources of nitrogen has been widely supplanted by the use of chemical fertilizers such as ammonia, ammonium salts, nitrates, urea, and urea derivatives. Legumes and non-legumes have been used for amenity planting and reclamation in Canada, but only on a small scale. Only about 206 000 actinorhizal non-legumes (eg. Elaeagnus spp., Alnus spp., etc.) were grown in government nurseries in 1978 compared with 7.4 million plants in USA (Fessenden 1979). Fertilization is by far the most widely used forest soil improvement tool, but in Canada even this technique is remarkably little used, although many forest soils suffer from nutrient deficiencies. About 10 000 to 15 000 ha^{-1} $year^{-1}$ of Canadian forests are fertilized each year, compared with 204 000 ha^{-1} $year^{-1}$ in Sweden and 91 000 ha^{-1} $year^{-1}$ in the USA (Burgess 1983). The high level of fertilizer use in Europe reflects the high intensity of forest management and the need to maintain yield. If forest management is intensified in Canada, much more active soil management, including fertilization and other techniques, will be necessary.

In the majority of cases, forest fertilization has been carried out empirically. Attention has been concentrated on the response of the trees (growth, leaf nutrient content, etc.), and little or no attention has been given to the effects on the soil biota. These effects are not fully understood for forest soils although more is known for the agricultural situation. On poor forest sites with high carbon:nitrogen ratios (typified by relatively acid forest soils with more humus), the application of nitrogen fertilizer can stimulate tree growth and the activity of soil microorganisms. Applying nitrate nitrogen can stimulate organic matter mineralization and nitrification with consequent loss of mobile nitrate and soil organic matter. Many agricultural soils have lost nitrogen and organic matter due to disturbance and heavy fertilization (Campbell et al. 1976).

Taking these factors into account, as well as the fact that the manufacture of nitrogen fertilizer needs a great deal of energy compared with other fertilizers (Table 7) and about 40% of applied nitrogen is lost as gases or by leaching (Konkles 1973, Knowles 1965), it is clear that, although fertilizers are a valuable management tool, they need to be used

Table 7: Energy required for the production of fertilizers (after Burgess 1983 and David and Blouin 1977)

Fertilizer	Energy per ton of nitrogen (kJ x 10^6)
Nitrogen Fertilizers	
Ammonia	44
Solid urea	52
Urea solution	47
Solid ammonium nitrate	53
Ammonium nitrate solution	49
Phosphate Fertilizers	Energy per ton P_2O_5 (kJ x 10^6)
Wet process acid	11
Triple superphosphate	12
Normal superphosphate	2
Potassium Fertilizer	Energy per ton K_2O (kJ x 10^6)
Potassium chloride	4

with caution. Account has to be taken of energy costs, the effects on the balance of soil elements, and their impact on soil processes concerned with mineralization and nitrification. Fertilizers are not the whole answer to maintaiing site productivity in intensive forestry systems, and biological techniques will be needed. Use of nitrogen-fixing symbioses appear to be a potentially powerful tool for the maintenance of soil organic matter and nitrogen levels.

4.0 THE OCCURRENCE OF NITROGEN FIXATION

4.1 Free-living organisms

Nitrogen-fixation by free-living organisms (Table 8) and symbioses (Tables 9 and 10) with higher plants occurs throughout the world, but is particularly active on poor sites in the Tropics. Aerobic, anaerobic, and facultative anaerobic nitrogen-fixing bacteria form a part of normal soil population, and the abundance and activity of these organisms depends upon such factors as energy supply, water status, and pH.

There are nitrogen-fixing bacteria that are adapted to acid soils (e.g. Beijerinckia which tolerates a pH of 4.0 to 5.0) while others, such as Azotobacter, are intolerant of acid conditions (Hendrickson et al. 1982). Wet, anaerobic conditions favour nitrogen fixation, and where both aerobic and anaerobic soil microsites are available a wide spectrum of both aerobic and anaerobic bacteria can fix nitrogen (Rice et al. 1967). Nitrogen fixation also occurs to a significant extent in the rhizosphere of plants (Richards 1973). Although free-living diazotrophic soil bacteria are very common and widespread, they are relatively inefficient compared with nitrogen-fixing systems associated with photosynthesis.

Free-living nitrogen-fixing phototrophs (which use sunlight energy to fix CO_2 by photosynthesis) (Table 8) have an in-built energy supply and are much more efficient nitrogen fixers than other free-living bacteria. The phototrophs include the aerobic blue-green algae mentioned earlier as well as anaerobic and faculative phototrophic bacteria. Anaerobic Chromatium and Chlorobium oxidize sulfur, sulfides, and sulfates, absorbing energy by chloropyll and carotenoid pigments and producing metabolites that can be used for both growth and nitrogen fixation (Postgate 1978).

The aerobic blue-green algae (which are true bacteria) are the most important phototrophs. They occur in Canadian forest soils and colonize disturbed areas, bare rocks, buildings, and many substrates of low fertility. They grow on tree trunks, leaf surfaces, and decaying organic matter and are the main aerobic nitrogen fixers in the oceans (Postgate 1978). The contribution of these ubiquitous, free-living, aerobic photographs should not be underestimated, but few data are available for their contributions to the forest nitrogen cycle.

4.2 Symbiotic associations

There are two main types of nitrogen-fixing symbioses; the legume-Rhizobium symbiosis and the non-legume actinorhizal symbiosis involving the actinomycete Frankia.

Legume symbioses are important in agriculture where they are used to improve soils and produce crops such as lentils (Lens), peas (Pisum), groundnuts (Apios), and forage (e.g., Medicago). Legumes also occur naturally on a wide range of sites, and both vetches (Vicia spp.) and wild clovers (Trifolium spp.) grow in forest clearings, on the edge of woodlands, and colonize stony sites and roadsides. Legumes are much more common in the

Table 8: Genera of free-living microorganisms which include nitrogen-fixing types (after Postgate 1978)

Strict anaerobes	*Clostridium* *Desulfovibrio* *Desulfomaculum*
Facultative[1]	*Klebsiella* *Bacillus* *Enterobacter* *Citrobacter* *Escherichia* *Propionibacterium*
Microaerophiles[2]	*Mycobacterium* *Thiobacillus* *Spirillum* *Aquaspirillum* *Methanosinus* *Rhizobium* (cowpea type)
Aerobes	*Azotobacter* *Azotococcus* *Azomonas* *Beijerinckia* *Derxia*
Phototrophs	
i Anaerobes	*Chromatium* *Chlorobium* *Thiopedia* *Ectothiospira*
ii Facultative	*Rhodopseudomonas* *Rhodospirillum*
iii Microaerophiles	*Plectonema* *Lyngbya* *Oscillatoria* *Spirulina*
iv Aerobes	*Anabena* *Nostoc* *Calothrix* *Gloeocapsa* etc.

[1] Grows with or without aerial oxygen when provided with fixed nitrogen, but fixes nitrogen only anaerobically.

[2] Sensitive to oxygen but can grow aerobically if the partial pressure of oxygen is low.

Table 9: Legume genera growing in the wild[1] state in Canada
(Nomenclature after Scoggan 1978)

Genus	No. of Species
Amorpha L.	3
Amphicarpa Ell. (Hog-peanut)	1
Anthyllis L. (Lady's Fingers)	1
Apios Medic. (Groundnut)	1
Astragalus L. (Milk Vetch)	c. 40
Baptisia Vent. (False Indigo)	c. 1
Caragana Lam. (Pea-shrub)[2]	2
Cassia L. (Senna)	c. 1
Cercis L. (Judas-tree)[2]	1
Coronilla L. (Crown Vetch)	2
Cytisus L. (Broom)[3]	c. 1
Desmodium Desv. (Tick Clover)	9
Genista L. (Dyer's Greenweed)	1
Gleditsia L. (Honey Locust)[2]	1
Glycyrrhiza L. (Licorice)	1
Gymnocladus Lam. (Kentucky Coffee Tree)[2]	1
Hedysarum L. (Sanfoin)	3
Lathyrus L. (Wild Pea)	14
Lespedezia Michx. (Bush Clover)	4
Lotus L. (Deer Vetch)	9
Lupinus L. (Lupin)	28
Oxytropis D.C. (Stemless Locoweed)	16
Psoralea L. (Scurf-Pea)	4
Robinia L. (Locust)[2]	3
Strophostyles Ell. (Wild Bean)	1
Tephrosia Pers. (Goat's Rue)	1
Thermopsis R. Br.	1
Trifolium L. (Clover)	20
Ulex L. (Furze)[3]	1
Vicia L. (Vetch)	10

[1] The list includes some non-native genera introduced from Eurasia that are well established as part of the Canadian flora. The list does not include introduced agricultural legumes that have not escaped and become established to a significant extent. The list is not complete.

[2] Tree or large shrub.

[3] Woody shrub.

Table 10: Genera of non-legumes with root nodules (after Fortin and Carlisle 1983).[5, 6]

Genus[1]	Family[2]	No. of[1] Species	Habit[2]	Distribution[2]	Use[1, 2]	N-fixation in some spp.
1. *Alnus* Ehr.	Betulaceae	25	Tree and shrub	N. Temp. Andes	Wood fibre	+
2. *Colletia* Comm. ex Juss	Rhamnaceae	15	?	S. America	?	+
3. *Discaria* Hook.	Rhamnaceae	15	Shrub	Andes, Brazil, N.Z., Australia	–	+
4. *Ceanothus* L.	Rhamnaceae	40	Shrub Understorey sp.	N. America	Ornamental[2] Shrub	+
5. *Shepherdia* Nutt.	Elaeagnaceae	3	Shrub Understorey sp. in Rockies	N. America	Some species[2] edible	+
6. *Hippophaë* L.	Elaeagnaceae	2	Shrub	N. Temp.	–	+
7. *Elaeagnus* (Tourn.) L.	Elaeagnaceae	30	Shrub	Asia, Europe	Edible Fruit[2] Cultured Shrub	+
8. *Coriaria* Niss. ex L.	Coriariaceae	10	Shrub	Mediterranean, Japan, N.Z., Chile, Mexico	Tan and Dye[2]	
9. *Purshia* DC	Rosaceae[4, 6]	1	Shrub	USA	Browse[1]	+
10. *Cercocarpus* H.B. et K.	Rosaceae[4, 6]	10	Shrub Small tree	Mexico, USA	Browse[1]	?
11. *Dryas* L.	Rosaceae[4, 6]	2	Shrub	Arctic, Alps	–	+
12. *Casuarina* L.	Casuarinaceae	35	Tree and shrub	Australasia	Wood fibre[2]	+
13. *Myrica* L.	Myricaceae	45	Shrub	Temp., N. Temp. Sub-tropical	–	+
14. *Comptonia* l'Her.	Myricaceae	1	Shrub	N. America	–	+
15. *Datisca* L.	Datiscaceae	2	Shrub	USA	–	+[3]
16. *Arctostaphylos* Adans.	Ericaceae	32	Shrub	N. Temp., Arctic	–	–

[1] Bond 1976, 1977, Burns and Hardy 1975, Fortin *et al.* 1979.

[2] Willis 1951.

[3] Winship and Chaudhary 1979.

[4] *Rubus* (*Rosaceae*) recently reported to form nodules (Akkermans and Houwers 1979).

[5] Species lists given in Bond 1976.

[6] *Cowania mexicana* var. *stansburyana* (Torr.) eps.) and *Chamaebatia foliolosa* (*Rosaceae*) are now known to form N_2-fixing symbioses (Righetti and Munns 1980, Heisey *et al.* 1980).

Tropics, where they include many trees and food crops of economic importance (National Academy of Sciences 1979). Altogether there are 18 000 species of legumes representing a wide range of genetic material from which geneticists can select. Many of the agricultural legumes only grow on well-illuminated sites and fertile soils with adequate levels of bases. Table 9 gives a list of legume genera growing in the wild in Canada.

The nitrogen-fixing symbioses of actinorhizal non-legumes with the Frankia symbiont are not so numerous, diverse, or widespread as the legumes and have received less attention. These actinorhizal symbioses, however, grow well on relatively poor forest soils and are pioneers on soils of very low fertility, such as those recently revealed by the retreat of glaciers (Ugolini 1968). The actinorhizae include alders (Alnus spp.), Comptonia sp., and Mycrica spp. (Table 10), many of which are associated with forests and disturbed sites (Fortin and Carlisle 1983).

4.2.1 The legumes

The Leguminosae are a very large and diverse family and the genera increase in numbers from north to south. In Canada there are relatively few genera growing wild (Table 9) compared with the Tropics, but many imported species, varieties, and forms are used in agriculture. Their value in forestry is limited because many members of the family have relatively high demands for bases and light.

The arborescent types which form large trees in Canada (Cercis L., Gleditsia L., and Robinia L.) are confined to the milder climate of southern Ontario and represent the northern end of the hardwood populations of the United States. The tall shrub Caragana arborescens Lam., which was introduced form Asia, is hardy over a large part of central Canada and is used as hedges and agricultural windbreaks on the Prairies. Ulex L. and Cytisus L., both of which are woody shrubs that were imported from Europe, have become naturalized in Canada, and grow in parts of the country with milder, oceanic climates.

The potential of woody, arborescent legumes for the production of forest fibre is mainly limited to locations in Canada with mild climates, but the herbaceous legumes could be used widely as green crops for forest soil amelioration. Trifolium spp. (the clovers) are frequently used in forest nurseries to maintain levels of soil nitrogen and organic matter.

Haines and De Bell (1979) listed many native and naturalized legumes of potential value in forestry programs in the United States, including some species important in agriculture. Their list of legumes is longer than the Canadian list, reflecting the milder climate of the southern and coastal areas of that country. Some of the genera they listed are known to grow in Canada (e.g., Amphicarpa L., Baptisia Vent., Cassia Lam., Desmodium Desv., Trifolium L., and Vicia L.) and are worth consideration for use in forestry.

Not all of the Leguminosae nodulate and fix nitrogen. The family contains three large sub-divisions, the Mimosaceae, the Caesalpiniaceae, and the Papilionaceae. The first two groups are mostly tropical and the latter

group contains tropical and temperate species (Scoggan (1978) does not differentiate these groups within the legumes of Canada). About 75% of the species in the Caesalpiniaceae do not form nodules, while most species in the Papilionaceae nodulate. The species of the Mimosaceae are intermediate in this respect (Richards 1976). Most Canadian legumes nodulate.

4.2.2 Actinorhizal non-legumes

The actinorhizal non-legumes with their Frankia symbionts are all perennial shrubs or trees and as such have received far less attention than the annual or biennial legumes that have made such a significant contribution to agriculture. Sixteen genera of non-legumes (Table 10) are known to nodulate, but one of these (Arctostaphylos Adans) has not been observed to fix nitrogen. Recently Datisca L. has been found to nodulate and fix nitrogen in symbiosis and doubtless more symbioses will be added in the future (Fortin and Carlisle 1983, Bond 1976, 1977, Burns and Hardy 1975, Fortin et al. 1979, Willis 1951).

The Rhizobium symbiosis is confined to one family (the Leguminosae) and members of this family permit infection by the bacterium due to inherited, specific physical and chemical characteristics of the legume roots. In contrast, nodulation and fixation by Frankia symbioses occur in many families (the Betulaceae, Rhamnaceae, Elaeagnaceae, Coriaceae, Rosaceae, Casuarinaceae, Myricaceae, and Datiscaceae) (Table 10). All of these families include members with the physicochemical configuration of roots that permit infection by Frankia, suggesting a high degree of diversity in this actinomycete.

The actinorhizal non-legumes occur in a wide range of climates, from arctic and north temperate (Dryas, Comptonia, Alnus) to the subtropical (Casuarina). Most genera occur in temperate zones and Myrica species occur in both north temperate and sub-tropical situations. The actinorhizal non-legumes occur to some extent in the southern hemisphere (e.g. Coriaria, Discaria, Casuarina) (Table 10).

There is, therefore, a very large and diverse gene pool of actinorhizal plants that is largely untapped and about which little is known. Some species undoubtedly have potential value in forestry. Actinorhizal non-legumes include arborescent forms of considerable potential in forestry both for soil improvement and fibre production, particularly within the genus Alnus Ehr.

4.2.3 Alders in Canada

The taxonomy of alders in Canada and the USA is complex and the nomenclature is littered with synonyms and misnomers. Some authors refer to one variant as a species and others call it a subspecies or variety, particularly with Alnus crispa (Ait.) Pursh. and A. rugosa (Du Roi) Spreng. populations with their wide distribution and great morphological variation. Some variants have been misidentified. Alnus rhombifolia Nuttall, for example, a 25 m tall tree growing on the Californian coast and in the dry, interior valleys of southwest Oregon (Johnson 1968a), was thought to be native in southern British Columbia, but Scoggan (1978) indicates that the Canadian populations are either Alnus crispa spp. sinuata (Regal) Hult. or A. rhombifolia of cultivated origin.

Scoggan (1978), in his comprehensive Flora of Canada, has simplified the taxonomy of Canadian alders (Table 11). Scoggan does not list the western thinleaf alder (A. tenuifolia Nuttall) as a separate species, but refers to it as Alnus crispa var. occidentalis (Dippel) Hitchcock. Similarly, Sitka alder (Alnus sinuata) (Regel) Rydberg is referred to as Alnus crispa ssp. sinuata (Regel) Hulten (Scoggan 1978, Johnson 1968a). According to Scoggan (1978) the most widespread Canadian alders are Alnus crispa (Ait.) Pursh. and Alnus rugosa (Du Roi) Spreng. and these species are mainly shrubs (Johnson 1968b, Clarkson 1960). Nevertheless A. rugosa also forms 10 m high trees and there may be arborescent variants of greater size. The vast populations of these alders have not been studied in detail, but both species are variable and likely to respond well to selection and breeding. The possibility of hybridizing the hardy, shrubby forms with arborescent native and introduced species should be considered. These genera have the advantage that they propagate fairly easily from seed and are not too difficult to grow in tissue cultures (Brown 1981).

Red alder (Alnus rubra Bongard) forms vigorous shrubs and trees more than 20 m high. It grows best on the moist, fertile soils of coastal British Columbia and California, reaching as far north as the Arctic circle on coastal sites. These alders invade cutover forests to the point of excess and foresters regard them with mixed feelings (Tarrant 1977). In close stands, or mixed with cottonwoods, the yield of red alder is very high and the fibre can be used for many purposes (Smith 1967, Briggs et al. 1978). In some areas red alder has high potential as a fibre producer and soil improver. Although there are provenances in Idaho (Johnson 1968b) that may well be hardy in areas with the continental climate of central Canada, red alder does not have a high potential in these colder areas, except as genetic material for breeding.

Some of the introduced arborescent alders grow well in Canadian situations. Alnus glutinosa (L.) Gaertn., the black alder of Europe and Asia, grows in southern Ontario, Nova Scotia, and Newfoundland, and appears to be naturalized on some sites (Scoggan 1978). In its native situation (western Europe to western Asia, northern Scandinavia to Asia Minor) it forms trees 20 m to 40 m tall and grows on a variety of moist soils. Although selection and testing have been carried out in Europe, the large northern populations of black alder are poorly understood and could yield suitable material for use in Canada either directly or by breeding (McVean 1953a,b, 1955, 1956a,b, Clapham et al. 1952).

Alnus incana (L.) Moench, the grey alder, is often shrubby, but sometimes forms 20 m high trees and grows on poor soils. Its natural population extends from northern Europe to the Italian Apennines (Clapham et al. 1952) and selection should yield material suitable for Canada.

Only six species of alder have been discussed above. Earlier this century only 25 species of Alnus were recognized by Willis (1951), but according to recent taxonomic analyses by Furlow (1974, 1979a, 1979b) there may be 200 or more species. The detailed analysis of Alnus by Furlow (1974, 1979a,b) and Murai (1964) differs from the classification used by Scoggan (1978). Furlow and Murai recognize three subgenera of alder i.e. Alnus,

Clethropsis, and Alnobetula. In this classification the Canadian species A. rugosa (Table 11) is called A. incana subspecies rugosa within the subgenus Alnus, while A. crispa is termed Alnus viridis subspecies crispa within the subgenus Alnobetula. Alnus rubra retains species status in the subgenus Alnus. It is suggested that for silvicultural purposes the simpler classification by Scoggan (1979) be used, although geneticists may prefer more detailed taxonomic differentiation.

The chromosome numbers of Alnus are discussed later in this review, but in passing it should be mentioned that while the general "normal" chromosome number of alders is usually recognized as n = 28, there is some evidence that the "normal" condition of alders is tetraploid (4n = 28), with a "basic" haploid n = 7, and an alder with a chromosome number 2n = 14 may be polyhaploid (Chiba 1966, Gram et al. 1941, Hall and Maynard 1979).

There are many alder species and variants scattered throughout botanical gardens and experimental plots in Canada and the United States. The Montreal Botanical Gardens, for example, include a small but interesting collection of Asiatic alders in the arboretum. The population of A. rhombifolia in the western USA is also of interest. If plans are made to use alders on any scale it would pay to examine, catalogue, and test these foreign genotypes for possible use in Canadian programs.

Table 11: The taxonomy of *Alnus* Ehrhart in Canada (after Scoggan 1978)

Family: *Betulaceae* Genus: *Alnus* (Alder, Aulne)					
Genus and species	Subspecies	Variety	Form	Maximum height (metres)	Habit
Alnus crispa (Ait.) Pursh. Green or mountain alder	*sinuata* (Regel) Hulten				Shrubs
	crispa	*mollis* Fern.			
		elongata Raup.			
		crispa	*stragala* Fern		
			crispa	c. 4	
Alnus rugosa (Du Roi) Spreng. Speckled alder	–	*serrulata* (Ait.) Winkler			Shrubs and small trees
		occidentalis (Dippel) Hitchcock			
		americana (Regel) Fern.	*hypomalaca* Fern.		
		rugosa	*tomophylla* Fern.		
			americana		
			emersoniana Fern.		
			rugosa	c.10	
Alnus rubra Bongard (Red alder; Oregon alder (U.S.))	–	*pinnatisecta* Starker	–	20	Shrub and tree

5.0 THE NITROGEN FIXATION PROCESS AND GENETICS

5.1 The process

Nitrogen fixation is the anaerobic reduction of molecular nitrogen gas (N_2) by hydrogen to ammonia by the enzyme nitrogenase, using ATP (adenosine triphosphate) energy.

The two nitrogen atoms in the dinitrogen (N_2) molecule are linked by a stable triple bond ($N \equiv N$) which requires a great deal of energy to disrupt. This stable triple bond renders the dinitrogen molecule relatively inert, unreactive, and unable to participate directly in the metabolism of higher plants.

The triple bond is responsible for the high energy cost of manufacturing nitrogen fertilizer (Table 7) compared with other fertilizers. In the industrial Haber-Bosch process the bond is disrupted by red hot magnesium or another catalyst at high pressures (Fortin and Carlisle 1983). The procedure uses natural gas as the hydrogen source, and natural gas, oil, and electrical energy for process heat.

Biological nitrogen fixation is able to carry out this energy-demanding reduction process at ordinary temperatures, provided an energy supply is available, because of the unique properties of the enzyme nitrogenase. The energy is provided by the hydrolysis of ATP to ADP (adenosine diphosphate) which releases energy.

In brief, the reaction is

$$N_2 + 3H_2 \xrightarrow[ATP \quad ADP]{\text{nitrogenase}} 2NH_3$$

Nitrogenase is inhibited by oxygen and low levels of oxygen are necessary for the reaction. The chemistry of nitrogen fixation is complex and more details can be obtained from reports by Postgate (1978) and Burns and Hardy (1975).

The enzyme nitrogenase has specific properties that include a degree of self-regulation (Postgate 1978):

i The enzyme consists of two proteins, azoferredoxin (a Fe-protein) and molybdoferredoxin (a MoFe-protein). The process can be limited by molybdenum and iron deficiencies.

ii Nitrogenase is destroyed by oxygen. The nitrogen-fixing microorganisms and the host plants have mechanisms for removal of oxygen from the site of enzyme activity.

iii The enzyme can convert ATP to ADP and release energy for use in nitrogen reduction by hydrogen.

iv The enzyme is inhibited by ADP, which provides a degree of self-regulation in the ATP ADP reaction.

v Nitrogenase not only reduces triple-bonded nitrogen gas, but can also reduce other triple-bonded molecules, an attribute that permits the assay of nitrogenase activity in the laboratory.

vi The enzyme reduces hydrogen ions (H^+) to gaseous hydrogen molecules. This can lead to hydrogen losses from the system, representing a waste of energy.

The ATP energy source consists of ATP molecules of the MgATP type; magnesium and phosphorus are essential to the process. The electon transfer process and MgATP appear to be linked to the azoferredoxin (Fe-protein) site where the MgATP is hydrolized to ADP. The molybdoferredoxin (the MoFe-protein), an important reduction site, is essential for the process because molybdenum is a specific catalyst for reduction. Nitrogen fixation appears to be optimal at a temperature of 25°C, and a neutral pH. Low temperatures can inhibit nitrogenase activity (Burns and Hardy 1975, Postgate 1978, Schrauzer 1977, Stiefel 1977, Fortin and Carlisle 1983).

Foresters considering using nitrogen-fixing symbioses in the management of forests will need to bear in mind this process, and the need for nutrient elements such as Mg, Mo, and P. The greatest potential for nitrogen-fixing symbioses in forest operation is on poor soils, but the nitrogen-fixing processes are likely to be inhibited if there are marked deficiencies of Mo, Mg, and P. On very poor soils it may well be necessary to use supplementary chemical fertilizers and to promote better phosphorus uptake by biological means (e.g. mycorrhizae) for the nitrogen-fixing symbioses to be effective.

The quantities of nitrogen fixed depend to a large extent upon the energy supplied. As mentioned earlier in this report, the free-living, non-photosynthetic organisms outside the rhizospheres rely their substrates such as soil organic matter for energy, and fix relatively little nitrogen. The supply of energy in the form of root exudates rich in carbohydrates can be considerable in the rhizosphere, but huge numbers of soil organisms congregate in that zone and nitrogen-fixing organisms must compete with them.

The free-living blue-green algae carry out photosynthesis which provides the nitrogen-fixing process with energy. They have the ability to fix considerable amounts of nitrogen (Table 6) in the upper zones of the soil, on plant leaves, and in aquatic environments. These organisms have not been studied much in Canada.

Nitrogen-fixing symbioses, where the microorganism forms an intimate association with photosynthesizing herbs, shrubs, and trees, supply the nitrogen-fixing process with energy in the form of photosynthates. This enables these symbioses to fix up to 300 kg ha^{-1} $year^{-1}$ of nitrogen (Table 6).

There can be hydrogen leakages in the nitrogen-fixing process which represent a waste of ATP energy as far as the plant-microorganism symbiosis is concerned. The emission of hydrogen means that not all the hydrogen from the breakdown of water attaches to nitrogen, and the end-product is of no metabolic use. Cell-free nitrogenase will evolve hydrogen if nitrogen, or a similar hydrogen receptor, is not available.

Some nitrogen-fixing bacteria have mechanisms to limit this hydrogen loss and others do not. Azobacter, for example, does not usually emit hydrogen because it contains a form of the enzyme hydrogenase that catalyses hydrogen uptake rather than hydrogen evolution. When Azotobacter's hydrogenase activity is inhibited by acetylene or carbon monoxide, its nitrogenase system wastefully emits hydrogen. In contrast, Klebsiella and Clostridium which both have low ATP efficiency, lack this type of hydrogenase system and evolve hydrogen during anaerobic growth (Postgate 1978).

Some of the legumes emit large quantities of hydrogen. The rhizobial symbioses of soya beans and garden peas are relatively inefficient nitrogen-fixers; 55% and 39% respectivley of their nitrogenase activity is diverted to wasteful hydrogen evolution. These hydrogen losses are so large that they have attracted the attention of scientists seeking a way to produce hydrogen fuel without large energy inputs from coal, oil, gas, or electricity, but little progress has been made (Postgate 1978, Fortin and Carlisle 1983). The actinorhizal Frankia symbioses do not seem to emit large quantities of hydrogen, but more information is needed. There appear to be opportunities for genetic selection and manipulation within the rhizobial symbioses to select strains with efficient nitrogenase systems.

The control of oxygen at the site of the anaerobic nitrogenase activity is complex, but has some bearing on future research opportunities. Each group of plants has developed its own strategy to allow just enough oxygen into the bacteria to permit multiplication and ATP formation without inhibiting the activity of the oxygen-sensitive nitrogenase enzyme. The blue-green algae photosynthesize and produce oxygen within the cells and oxygen must be prevented from reaching the nitrogenase site.

Oxygen levels are lowered in some cases simply by respiration. Some nitrogen-fixing organisms, such as Clostridium pasteurianum and Desulfovibrio desulfuricans, reduce sulphates and avoid the oxygen problem. Some bacteria, such as the aerobic Beijerinckia, form coats of polysaccharide slime on the cells to impede oxygen entry to the nitrogenase site. In some cases nitrogenase seems to be capable of adopting a molecular conformation that is inactive; when oxygen levels fall the enzyme reverts to the active form. Photosynthesizing blue-green algae form heterocysts along algal filaments, isolating nitrogenase from the photosynthesizing, oxygen-emitting zones on the filaments (Postgate 1978, Chatarpaul and Carlisle 1983).

The oxygen exclusion system of rhizobial legumes consists of a haemoprotein similar to haemoglobin. The legume haem compound has a high affinity for oxygen and manages to control oxygen tensions at the site of

nitrogenase activity, so as to provide sufficient oxygen for the metabolism of the bacteria without damaging the nitrogenase enzyme. The host plant interacting with the Rhizobium is responsible for the production of haemoglobin, and fast-growing rhizobia do not appear to be able to fix nitrogen in vitro in isolation and seems to be host dependent. Some slow-growing rhizobia appear to be able to fix nitrogen outside the host plant, but it is necessary to lower oxygen levels (Postgate 1978). This bacterium-host plant dependency due to haem production by the host means that many more genes are involved than would be the case if the microorganisms were autonomous for oxygen control and nitrogenase activity. That fact has made it difficult for geneticists to transfer the Rhizobium to plants other than legumes to form nitrogen-fixing symbioses. Not only has the host's infection barrier to be overcome, but haem production must be maintained, involving the manipulation of a large number of genes.

The situation is simpler in actinorhizal non-legume symbioses involving the actinomycete Frankia (e.g., Alnus spp., Myrica spp. etc.). Frankia appears to be autonomous for oxygen exclusion and able to perform away from host plants. This has decided advantages for genetic manipulation because the host's haem producing systems need not be taken into account (Fortin and Carlisle 1983).

5.2 Genetics

Bacteria (such as Rhizobium) possess a circular, gene-bearing chromosome and one or more gene-bearing plasmids that are much smaller than the main chromosome. The genes responsible for nitrogen fixation and its regulation are called the nif cluster. On Klebsiella, for example, the nif cluster is on the main chromosome, between genes concerned with the uptake of shikemic acid (shi) and synthesis of histidine (his). Postgate (1978), in a simplified outline of the genes, states that the nif cluster consists of two genes (K and D) coded for the production of nitrogenase's molybdenum-protein, one gene (H) for the production of the iron-protein, and three regulatory genes (E, A, and L). There are also F and B genes concerned with nitrogenase reduction and formation of part of the molybdenum-protein respectively. More recently it has been found that there are 17 nif genes, but the function of five of them is unknown (Stewart 1982). While the nif genes of Klebsiella are on the main chromosome, there is evidence that in Rhizobium, the legume symbiont, the nif genes are located on large plasmids together with the genes coding for nodulation and host specificity (Stewart 1982). The presence of plasmids in Frankia was demonstrated by Normand et al. (1983).

Plasmids allow a bacterium to transfer genetic material (DNA) to another bacterium by pseudosexual conjugation, so that active nif genes can be transferred to bacteria that do not normally fix nitrogen. The nif genes can also be transferred to new bacterial hosts by bacteriophages (bacterial viruses) (Postgate 1978).

As mentioned earlier, although it is possible to transfer nif genes to free-living bacteria that will subsequently fix nitrogen, this does not mean that the bacteria will necessarily form nitrogen-fixing symbioses with new

hosts. Transfer of a nitrogen-fixing symbiosis from one species of higher plant to another is difficult due to the necessity of overcoming the host's infection barrier and the need to develop an oxygen regulation system in the new host if the microorganism (e.g. Rhizobium) is not autonomous for that attribute.

There is evidence of specificity in both legumes and actinorhizal non-legumes. Legumes have been divided into seven groups. Within each group plants form nodules with the same Rhizobium strain, but do not usually nodulate with the bacterial strains from other groups. The seven groups are the field pea (Pisum), medic (Medicago), clover (Trifolium), lupin (Lupinus), garden pea (Phaseolus), soybean (Soya), and cowpea (Vigna), and within these groups there are highly specific sub-groups. The Rhizobium strains differ in their ability to form nodules with particular legumes and in their nitrogen-fixing ability. A Rhizobium strain that is highly effective in one host may behave poorly on another host because nitrogen fixation is affected by both host and bacterium (Richards 1976).

The Frankia symbiont in actinorhizal non-legumes also exhibits some degree of specificity, but not to the extent of the Lequminosae. Frankia from one genus may not infect plants of another group, and inocula from Hippophaë rhamnoides and Coriaria myrtifolia do not form nodules on Alnus glutinosa. Elaeagnus may weakly nodulate alder, and while Myrica inocula can nodulate alder, the number of nodules per plant is low (Rodriguez-Barrueco 1979) (Table 12). The actinomycete in Comptonia peregrina nodules will not inoculate Alnus glutinosa seedlings, but will inoculate Alnus cordata, A. crispa, A. rugosa, A. rubra, and A. serrulata seedlings (Lalonde and Calvert 1979). There is evidence of incompatibility within genera (e.g. Coriaria) (Richards 1976).

Several soil bacteria appear to be involved in the nodulation of red alder with the Frankia actinomycete from Comptonia peregrina (strain CpI1). Knowlton et al. (1979) found that when aseptic seedlings of red alder were grown on dilute Hoagland's agar on slants in sterile test tubes, the plants failed to nodulate when infected by the Comptonia actinomycete CpI1. Nodulation after inoculation by Frankia occurred in the presence of four assisting bacteria (all of which were gram-negative motile rods). The nodules contained the actinomycetes with vesicles. The pure CpI1 Frankia strain also failed to nodulate Comptonia peregrina or Myrica gale in stringent, axenic culture. It appears that, at least for some actinomycete strains, "helper" organisms are necessary to secure nodulation, illustrating the complex interrelationships of microorganisms in the rhizophere. More recently, Perinet and Lalonde (1983) were able to successfully inoculate Frankia on Alnus crispa seedling obtained from tissue culture under aseptic conditions. Foresters contemplating using actinomycorrhizal non-legumes will need to take care in the selection of compatible, effective actinorhizal inocula.

The evolution of nitrogen fixation is a point in question. Although there are traces in Precambrian rocks (over 3×10^9 years old) of organisms that resemble heterocystous blue-green algae, and nitrogen fixation is frequent in the relatively primitive anaerobic bacteria, Postgate (1978)

Table 12: Effect of inocula made from nodule material from different sources on nodulation of six actinorhizal non-legumes[1] (Compiled from Rodriguez-Barrueco and Miguel 1979)

Host plant	Inoculation source	% Nodulated	Mean no. of nodules per plant	Nitrogen fixation
Alnus glutinosa	*Elaeagnus angustifolia*	57	5	+
	Hippophaë rhamnoides	0	–	–
	Coriaria myrtifolia	0	–	–
	Myrica faya	40	3	?
	M. gale	100	20	+
Elaeagnus angustifolia	*Alnus glutinosa*	14	–	?
Coriaria myrtifolia	*Alnus glutinosa*	0	–	–
	Hippophaë rhamnoides	100	22	+
	Myrica faya	100	12	+
	M. gale	100	14	+
Myrica faya	*Alnus glutinosa*	100	2	+
	Coriaria myrtifolia	100	3	+
	Hippophaë rhamnoides	100	2	+
	Myrica gale	100	2	+

[1] Inocula obtained by immersion of roots in a crushed nodule suspension. Plants grown in nitrogen-free Crone solution with pH 5.4 for *Alnus*, 7.2 for *Coriaria* and 6.3 for *Myrica*.

Postgate (1978) suggests that this does not necessarily indicate that nitrogen fixation developed a very long time ago. He queries the benefit of nitrogen fixation in early times when the Earth's atmosphere contained little oxygen but some free ammonia. Also, there has not been an appreciable degree of evolutionary divergence in the long period since ammonia in air virtually disappeared. Postgate (1978) suggests that nitrogen fixation, which can be carried out by aerobes as well as anaerobes, could have emerged relatively late in evolutionary history, after oxygen appeared in the atmosphere and plants had used up superficial supplies of combined nitrogen (ammonia, nitrates, etc.). If this is the case, nitrogen fixation may be at an early stage in its evolution. The Precambrian "heterocysts" could possibly have been spore-like bodies not associated with nitrogen fixation.

There are major opportunities for the selection and breeding of both higher plants such as alders (that are known to be able to form nitrogen symbioses) and microbial symbionts such as Frankia. The selection of alders and Frankia was part of the ENFOR-supported research and will be described later in this report.

5.3 The measurement of nitrogen fixation

Nitrogen fixation rates in a forest can be measured by compiling a stand budget and estimating nitrogen accretion. This technique has a drawback in that it involves estimating the total stand nitrogen at two points in time, and each estimate is usually approximate and often has a high variance. Significant differences need to be interpreted with caution (Carlisle 1980).

On a smaller scale, biological nitrogen fixation can be measured directly or indirectly. Direct methods include measuring nitrogen gas (N_2) uptake manometrically or measuring the production of ammonia from the reduction of nitrogen by hydrogen, but these methods are slow. Analysis of the stable isotope of nitrogen ^{15}N is a reliable method used to detect nitrogen fixation and is still the conclusive test for reduction of nitrogen to ammonia, but it is costly and relatively slow to use (Bergerson 1980).

A lot of information about rates of nitrogen fixation has been obtained since the indirect method involving acetylene reduction was developed. The enzyme and nitrogenase can reduce molecular nitrogen (N_2), nitrous oxide (N_2O), cyanide (CN^-), and acetylene (C_2H_2), and a technique was developed to assess nitrogenase activity by measuring rates of acetylene reduction by the enzyme using a gas chromatograph. The method is relatively simple and rapid, and apart from the cost of the chromatograph, is more economical than the direct methods (Turner and Gibson 1980). The acetylene reduction method has a drawback in that rates of acetylene reduction *in vitro* may not be precisely the same as the rate of reduction of nitrogen by nitrogenase in, for example, a plant nodule. It is necessary, therefore, to interpret results from the acetylene reduction method with some caution and to support them with ^{15}N assays when necessary. The methods of measuring biological nitrogen fixation have been fully reviewed in Bergerson (1980).

6.0 QUANTITIES OF NITROGEN FIXED

The quantities of nitrogen fixed by free-living organisms in forest soils on a global scale is about 10 kg ha^{-1} $year^{-1}$, but this includes the tropics where blue-green algae are sometimes very active. In temperate forests (other than alder woodland where special soil conditions and bacteria populations develop) the non-symbiotic nitrogen fixation is generally regarded to be about 2 to 5 kg ha^{-1} $year^{-1}$ (Table 6). This may be correct in very broad terms, but relatively little is known about nitrogen fixation by organisms in the rhizosphere, and there is evidence that, quite apart from the rhizosphere effect, rates of non-symbiotic nitrogen fixation may be appreciably higher in anaerobic soils.

Non-symbiotic nitrogen fixation was studied in aerobic and anaerobic conditions in the different horizons of mull, mor, and muck soils in Quebec (Knowles 1965, Chang and Knowles 1965). The non-symbiotic nitrogen fixation ranged from negligible to 9.3 kg ha^{-1} $year^{-1}$ of nitrogen on mull and mor soils, but fixation was as high as 55.2 kg ha^{-1} $year^{-1}$ in the A_1 horizon of the mull soil (Table 13). These values were obtained by extrapolation of data from a short-term (29 day) experiment, the data adjusted for assumed mean monthly temperatures and Q_{10} of 2. The results need confirming in longer-term experiments but, nevertheless, indicate the need to exercise caution in assuming very low levels of non-symbiotic nitrogen in forest soils. Denitrification by anaerobes can take place in anaerobic niches in the aggregates of apparently "aerobic" soils, and it is possible that non-symbiotic fixation occurs in similar anaerobic microsites and in plants' rhizospheres. It seems likely that using an overall estimate of 5 kg ha^{-1} $year^{-1}$ for non-symbiotic nitrogen fixation could lead to underestimates on relatively fertile forest soils such as mulls.

The data summarized in Table 6 indicate that both legumes and non-legumes can fix quantities of nitrogen (e.g. 2 to 300 kg ha^{-1} $year^{-1}$) equivalent to a heavy fertilizer dressing, and that fixation of nitrogen in some forests is very low (0.4 kg ha^{-1} $year^{-1}$). In the Tropics the fixation of 35 kg ha^{-1} $year^{-1}$ of nitrogen in rice paddies reflects the nitrogen fixation by photosynthesizing blue-green algae.

The data in Table 6 were obtained in several ways including estimating nitrogen accretion over defined periods, acetylene reduction assays, and green-house accretion per gram of nodule times the mass of nodules per unit area of land (Binkley 1981). The first two of these (accretion and acetylene reduction) have drawbacks described in Section 5.3, and care needs to be taken in interpreting estimates of nitrogen fixation based on nodule mass in the field. Nodule mass per hectare varies a great deal, for example, from 30 to 250 kg ha^{-1} in red alder stands aged 2 to 30 years and up to 450 kg ha^{-1} in Alnus glutinosa stands aged 20 years (Binkley 1981). Quite apart from the spatial variation, the relationships between nodule weight and quantity of nitrogen fixed in the field is a point in question, particularly in actinorhizal species where perennial nodules can be of different ages and vary in efficiency. Consequently, many of the field estimates of nitrogen fixation should be regarded as approximations.

Table 13: Non-symbiotic nitrogen fixation in mull, mor, and muck soils in Quebec (Abstracted from Knowles 1965, Chang and Knowles 1965)

Soil Type	Soil Horizon	Nitrogen fixation[1] (kg ha $^{-1}$ year $^{-1}$) Aerobic[2]	Anaerobic[2]
Mull	L + F	–	0.1
Mull	A_1	–	44.0
Mull	A_1	5.0	55.2
Mor	L	–	0 8
Mor	F(-H)	–	0.9
Muck (a)	–	7.0	19.8
Muck (b)	– [3]	9.3	39.0

[1] Calculated from rate of nitrogen fixation per gram of soil over a 29-day period.
[2] Corrected for temperature variations assuming a Q_{10} of 2.0.
[3] 1% glucose added.

7.0 THE ECOLOGY OF NITROGEN FIXATION

Nitrogen-fixing, free-living organisms and symbioses in forests compete with other organisms for food, water, light, etc. and participate in the cycling of nutrients, water, and energy. The soil is a dynamic, living system with huge numbers of organisms ranging in size from bacteria to large earthworms involved in the soil processes. The microbiota includes lower protists such as bacteria and blue-green algae, protozoa, and fungi. The meso- and macrobiota include a wide range of soil animals such as flatworms, nematodes, snails, slugs, earthworms, woodlice, millipedes, centipedes, springtails, mites, spiders, and insects such as ants, flies, beetles, etc. (Tables 14 and 15). All of these organisms interact either directly or indirectly with the nitrogen-fixing bacteria (including blue-green algae) and with each other (Richards 1973, Postgate 1978, Fortin and Carlisle 1983).

There are large numbers of organisms on the parts of forest ecosystems above the ground (trees, lesser vegetation, litter, fauna) and these organisms participate in the cycling of nitrogen. Casual, free-living associations of microorganisms occur on surface litter, tree bark, and branches, and leaf surfaces provide microhabitats for many lower and higher protists (bacteria, true algae, fungi, etc.), some of which are known to fix nitrogen (Jones 1976). Rotting logs contain diazotrophic bacteria that provide combined nitrogen for the metabolism of fungal mycelia involved in lignocellulose decomposition (Cornaby and Wade 1973). Lichens, consisting of fungus + blue-green alga symbioses, grow on the soil surfaces, rocks, tree stems, and other nutrient-deficient substrates, and some of these symbioses fix nitrogen. Some thalloid liverworts growing on moist shaded forest microsites have Nostoc, a blue-green alga, on their undersides and there is some evidence that this organism provides the host with combined nitrogen.

In the Tropics there are nitrogen-fixing symbioses between blue-green algae and water ferns (e.g. Azolla + Anabena), cycads (e.g. Macrozamia + Nostoc), and angiosperms (Gunnera + Nostoc). The Azolla + Anabena symbiosis is successfully used as a green manure in rice culture. Azolla grows in lakes in the temperate zone, but none of the other symbioses above occur in Canadian forests (Postgate 1978).

There are also associative symbioses between higher plant and microorganism partners that exhibit mutual interdependence, with each partner able to grow satisfactorily on its own. Most of these associative symbioses occur on grasses (Gramineae). For example, in the Paspalum notatum + Azotobacter paspali association, the Azotobacter forms a mucilagenous sheath on the grass roots. Another example is the Digitaria + Spirllum association within the tissues of the grass roots. Most of these associative symbioses on grasses occur in the Tropics, but some temperate weeds (e.g. Stachys sylvatica L. and Aegopodium podagravia L.) give positive acetylene tests, suggesting nitrogenase activity. However, the relationships between hosts and microorganisms may be casual in these cases (Postgate 1978). There is some evidence of appreciable nitrogen-fixing activity in beds of mosses such as sphagnum (Granhall and Lindberg 1978, Stewart 1982).

Table 14: The numbers of soil animals in mull or mor forest soils (compiled from Bornebusch 1939)

	Tree cover: Beech (*Fagus*) Acid raw humus (Mor) (pH 3.6 to 5.6)	Tree cover: Beech (*Fagus*) Mull soil (pH 5.8 to 6.1)
Species	Numbers in millions per hectare	
Earthworms (Oligochaetes)	0.8	1.78
Potworms (Enchatraeids)	7.81	5.34
Gasteropods (snails, slugs, etc.)	0.52	1.04
Millipedes	0.40	1.78
Centipedes	0.20	0.79
Mites and Springtails	112.39	44.09
Diptera and Elateridae larvae	13.09	2.45
Others (insects, isopods, spiders)	6.42	4.72

Table 15: Numbers of soil microorganisms in two Canadian podzolic soils (condensed from Timonin 1935)

Profile	Horizons (cm)	Bacteria	Actinomycetes	Fungi	Algae	Protozoa
		(x 1000 per gram)				
1	A_0 (0-10)	16,915	1,335	205.0	0.5	0.04
	A_1 (10-12)	73,000	16,000	65.0	5.0	0.02
	A_2 (12-20)	7,650	950	7.5	0.1	0
	B (20-50)	9,750	1,250	14.75	0.1	0
	C (50-100)	463	97	1.85	0	0
2	A_0 (0-9)	28,300	5,700	242.5	1.0	0.10
	A_1 (9-12)	4,500	3,500	20.0	0.1	0.02
	A_2 (12-20)	1,550	950	1.62	0	0
	B (20-55)	3,300	900	10.63	0	0
	C (55-100)	1,142	120	1.47	0	0

A growing body of evidence suggests that nitrogen-fixing species of bacteria inhabit the intestines of many mammals and insects, including those that occur in forests (French 1980, Fortin and Carlisle 1983).

There are, therefore, many opportunities for microorganisms with or without their plant hosts to fix nitrogen in forest ecosystems with their diverse microhabitats. Little work has been carried out on the quantities of nitrogen fixed by diazotrophic bacteria on ground vegetation, apart from the Frankia and Rhizobium symbioses. The loose associations and minor symbioses may make small contributions of combined nitrogen individually, but could be collectively significant on the time scale of conventional forestry rotations (more than 60 years).

7.1 Microorganisms in the soil biota

The microorganisms involved in forest soil processes are mainly bacteria (including actinomycetes and phototrophs) and fungi. The prokaryotic bacteria, which do not possess true nuclei, appeared on Earth about 3×10^9 years ago, and evolved in an oxygen-free atmosphere. Many bacteria are still anaerobic, but facultative and aerobic types have evolved that occupy a multitude of microniches in the soil. Bacteria in forest soils include types that are involved in the breakdown of complex soil organic matter to ammonium (NH_4^+) and nitrate NO_3^-) ions that vegetation can use, and also in the complex process of nitrogen fixation (Hendrickson et al. 1982). The fungi, which are eukaryotes (possessing nuclei and mitochondria), evolved only 450 million years ago and most of them are aerobic.

The bacteria and fungi interact in the ecosystem. Fungi and bacteria (i.e. blue-green algae) form symbioses in lichens, and some of these associations fix nitrogen. Mycorrhizal fungi and gram-negative bacteria form loose relationships in the rhizosphere where nitrogen-fixing bacteria occur and some of the rhizosphere actinomycetes are antagonistic to the fungal hyphae. The amoebae, ciliates, and flagellates browse on both fungi and bacteria and recycle their components (Hendrikson et al. 1982).

7.1.1 Soil bacteria

The types of bacteria, including those involved in nitrogen-fixation, vary from one forest soil to another. Flavobacterium, Chromobacterium, Corynebacterium, Arthrobacter, and Nocaridia are common in mull soils under broadleaved trees, Bacillus species are more common in relatively acid soils under conifers, and Arthrobacter is the predominant bacterium of agricultural soils. For many years actinomycetes (which include nitrogen-fixing types) were considered to be intolerant of acid conditions, but there is a group of actinomycetes that includes types living in relatively acid (pH 4.0) peats and organic forest soils (Jensen 1928). As many as 6 million actinomycetes per gram of soil have been recorded in the organic matter of podzols under pines (Pinus) (Corke and Chase 1964). About 56% of the bacteria in soils under alder + conifer mixtures in the Pacific Northwest consist of actinomycetes, and even under conifers they accounted for 45% of the bacteria (Lu et al. 1968). Little is known about the ecology and metabolism of these free-living actinomycetes, but they have a high requirement for cobalt, molybdenum, and citrate and this has been

interpreted to imply that they may fix nitrogen (Hendrickson et al. 1982). Actinomycetes inhibit Azotobacter colonies, many strains of which fix nitrogen and attack resistant organic compounds such as humic acids, chitin, and lignocellulose (Lacey 1973, Crawford 1978, Hendrickson 1982).

7.1.2 Soil fungi and mycorrhizae

Although it is known that soil contains a great many fungi, the abundant basidiomycetes in the soil do not readily culture and fruit in vitro, and it is difficult to assess their numbers and diversity. The Phycomycetes and Fungi Imperfecti produce many spores and culture relatively easily. Their mycelia are ephemeral in the soil unlike basidomycete hyphae (including mycorrhizae) which may persist for long periods (Hendrickson 1982).

Many fungi form mycorrhizal associations with higher plants. Endomycorrhizae occur on some broadleaved tree genera such as Acer (Tourn.) L., Alnus (Tourn.) L., Fraxinus Tourn. ex L., Liquidambar L., Liriodendron L., Platanus (Tourn.) L., Prunus (Tourn.) L., and Robinia L., but in some cases their identities are not clear. Vesicular arbuscular (VA) endomycorrhizae are formed on herbs, trees, and shrubs by fungi of the family Endogonaceae (Gigaspora, Glomus, and Acaulospora spp.).

Ectomycorrhizae occur on woody plants in the Betulaceae, Salicaceae, Tiliaceae, Ulmaceae, Rosaceae, and Leguminosae, and all conifers in the Pinaceae. Fungi involved in these ectomycorrhizal associations include ascomycetes, gasteromycetes, and basidiomycetes. Many of the colorful sporocarps seen in forests, such as those of Amanita, Russula, and Boletus spp., are parts of hyphal networks attached to ectomycorrhizal symbioses on the surrounding trees. The identities of mycorrhizae associated with actinorhizal nitrogen-fixing symbioses are part of the ENFOR-funded research at Laval University, Quebec, and will be discussed later in this report.

Both endomycorrhizae and ectomycorrhizae occur on the roots of actinorhizal non-legumes such as alders. In addition, the nodules on the roots contain nitrogen-fixing Frankia-alder symbioses, representing a four-way multiple symbiosis (the ectomycorrhizal symbiont + endomycorrhizal symbiont + actinorhizal symbiont + the host). This is further complicated by the interrelations between bacteria and fungi in a highly populated, nutrient-rich rhizosphere where fixation by associative free-living bacteria also takes place.

The full extent of these complex interactions is not fully known, but it is clear that mycorrhizae, particularly ectomycorrhizae with their extensive hyphal networks, play an important role in the symbiotic, nitrogen-fixing process on poor soils. Many forest soils are deficient in available phosphorus either due to a lack of phosphate minerals or to the phosphate being in a form not available to tree roots. The pervasive mycorrhizal hyphae explore soil microniches and have the ability to make better use of bound phosphorus than tree roots, and to provide the alder with phosphorus which is vital in the nitrogen-fixing process involving adenosine triphosphate (ATP) energy. There is also evidence that mycorrhizae provide a carbohydrate bank for times of stress, and improve both plant water relations and resistance to some diseases. The mycorrhizae

are closely linked with the processes of the diazotrophic actinomycete, the host, and nitrogen fixation (reviewed in Fortin and Carlisle 1983).

The mycorrhizae of alders are attached to a network of hyphae in the soil and it is possible that they provide pathways for the transfer of nitrogen fixed by the Frankia-alder symbiosis. The role of mycorrhizae in nitrogen transport is one of the topics addressed by the ENFOR-funded research at Laval University and this report will discuss it later.

7.1.3 Mineralization, nitrification, and denitrification

The processes of organic matter mineralization, nitrification, and denitrification are vital to the forest cycle. Microbial decomposition of soil organic matter releases ammonia (NH_3) as a waste product which may be lost from the system as a gas, or remain in the system in the NH_4^+ form. If nitrification is subsequently carried out by nitrifying bacteria, the product of the process is nitrate (NO_3^-). Firstly Nitrosomonas oxidises ammonium (NH_4^+) ions to nitrite (NO_2^-) and secondly Nitrobacter oxidises nitrite (NO_2^-) to nitrate (NO_3^-):

$$(i) \quad 2NH_4 + 3O_2 \xrightarrow{\text{Nitrosomonas}} 2NO_2 + 2H_2O$$

$$(ii) \quad 2NO_2 + O_2 \xrightarrow{\text{Nitrobacter}} 2NO_3^-$$

The nitrifying bacteria grow slowly and energy yields are low. The optimum pH for the activity of these bacteria is 7.5 to 8.0, and nitrate production is usually negligible at pHs of 5.0 or below. This varies from soil to soil, and there are strains of bacteria that carry out nitrification at pHs as low as 4.0. In acidic alder soils nitrification by these acidophilic strains of bacteria can be significant (Bollen and Lu 1968).

In general, nitrification is not regarded as very active in coniferous forest soils but more information is needed. It is possible that it has been underestimated.

If nitrification produces nitrate (NO_3^-), this mobile anion may be lost from the soil by leaching or uptake by vegetation. Negatively charged nitrate ions (NO_3^-) may move down the soil profile away from the aerobic upper zones to lower levels in the soil where anaerobic microsites are more plentiful. In these anaerobic situations the nitrate may be reduced by many species of denitrifying bacteria (Knowles 1982), mainly within the general Pseudomonas, Achromobacter, and, to a lesser extent, Denitrobacillus. The products of denitrification are nitrogen gas (N_2) and water (equation iii and iv); if the nitrate concentration is high the denitrifying bacteria may produce nitrous oxide (N_2O) (Richards 1976).

$$(iii) \quad NO_3^- + H_2 \longrightarrow NO_2^- + H_2O$$

$$(iv) \quad 2NO_2^- + 4H_2 \longrightarrow N_2 + 4H_2O$$

Following nitrification and denitrification there is a potential loss of nitrogen in the form of leached nitrate (NO_3^-) and gases (N_2 and N_2O). The released nitrogen (N_2) can, of course, be reincorporated in to the system by free-living bacteria or symbiotic associations with active nitrogenase systems (Hynes and Knowles 1980).

It is well known that after tree harvesting there is a flush of nitrate (NO_3^-) production due to the cessation of nutrient uptake by the trees, organic matter mineralization, and nitrification (Bromann et al. 1968), and the numbers of Nitrosomonas and Nitrobacter may increase as much as 18 and 34 fold respectively in cut areas (Smith et al. 1968). There is some evidence that mycorrhizae interact with soil microorganisms and suppress litter decomposition, thereby controlling excessive leaching and gaseous losses from nitrification and denitrification (Gadgil and Gadgil 1974, Romell 1935, 1938). The removal of inhibitory mycorrhizae may play a role in nitrogen mobilization following clear-cutting (which would destroy the mycorrhizal symbioses).

7.1.4 The rhizosphere

Large numbers of soil organisms, including bacteria, occur in the rhizosphere. Rhizosphere bacteria (including actinomycetes) consist of ammonifiers, nitrifiers, nitrogen fixers, and microorganisms vital to the nitrogen processes. These all live adjacent to mycorrhizae, Rhizobium nodules, and actinorhizae. The free-living nitrogen-fixing bacteria in the vicinity of the roots feed on root exudates, and it has been suggested that mycorrhizae provide a favourable environment for nitrogen-fixing soil organisms such as Klebsiella and Bacillus (Postgate 1978). Wollum and Davey (1975) suggested that nitrogen fixation in the rhizosphere in forests have received too little attention, and Bacillus polymyxa, the main free-living nitrogen-fixing bacterium in many acid forest soils, is very active in the rhizosphere. It is possible that the contribution of rhizosphere bacteria to the income of combined nitrogen in forest soils has been underestimated, and that rhizosphere fixation may be responsible for unexplained gains of nitrogen, as high as 50 kg ha^{-1} $year^{-1}$, recorded under some grass swards and conifers (Postgate 1978). As Richards (1973) points out, it seems unlikely that direct absorption of atmospheric ammonia is responsible.

7.1.5 Competition in the soil biota

The microorganisms and roots of higher plants compete for nutrients in the soil. The best known example is the addition to the soil of organic matter that contains little nitrogen, when bacterial and fungal uptake and immobilization of nitrogen can induce a nitrogen deficiency in higher plants. Many compounds are produced by both microorganisms and higher plants that inhibit other organisms (Hendrikson 1982). Some inhibitors are simple compounds such as ammonia (NH_3) released from mineralization of organic matter (Richards 1976). Other inhibitors are complex organic compounds such as antibiotics, resins, tannins, and phenols; the antibiotics can be effective at low concentrations. Actinomycetes (which include nitrogen-fixing genera) are particularly active in the production of antibiotics and have been used in the pharmaceutical industry. Bacillus spp. and Pseudomonas are also involved in antibiosis.

Fungi produce potent antibiotics, particularly members of the Fungi Imperfecti (e.g. Penicillin, Trichoderma, Aspergillus, and Fusarium), and these compounds greatly affect the number, activities, and succession of soil microorganisms, including mycorrhizae and nitrogen fixers. One example is the inhibition of the fungus Mortierella pusilla, an actomycorrhizal fungus on Tilia (Richards 1976, Park 1970). Antibiosis is usually of short duration since antibiotics can be degraded by other components of the soil biota or become deactivated by adsorption on soil colloids (Richards 1976).

Soil organisms are also regulated by leachates from the branches and foliage of higher plants such as trees, the litter from these higher plants, and root exudates. Alder produces many organic compounds that have a considerable impact on the soil biota, including phenols, fatty acids, and amino acids (Tarrant and Trappe 1971). The phenols in particular directly inhibit microorganisms and stabilize leaf protein substrates (Handley 1961).

Nitrogen-fixation by the actinorhizal symbiosis in red alder stands results in a high proportion of soil nitrogen being in the nitrate form, which is to the competitive advantage of soil bacteria with nitrate reductase systems and not to the advantage of soil organisms that lack it. The secretions from alder roots are responsible for characteristic alder rhizosphere populations developed in response to both high levels of phenolics and nitrogen. Alder-induced soil properties are considered to have potential for biological control of diseases such as the conifer root pathogen Poria weirii; red alder's phenolic exudates are known to inhibit Poria (Tarrant and Trappe 1971). Alder leaf litter has a marked effect on soil organic matter, pH, and nitrification. Bollen and Lu (1968) demonstrated in western USA that nitrogen levels and transformations are more rapid in soils under alder canopies than in soils under conifers; nitrification is particularly rapid in the F-layer of soils under alder despite lowered pH. Ammonification was also found to be relatively rapid in the A horizon of soils under red alder soils. Lu et al. (1968) found that soil under red alder contained large numbers of Streptomycetes, ranging from 10% of total bacteria in March, to 52% in July, and 35% in September. These bacteria produce antibiotics and could effect other organisms profoundly.

Red alder on sites in the western USA tends to lower soil pH by about one unit, increase absolute soil nitrogen content, lower levels of the cations Mg^{++} and Ca^{++}, and increase cation exchange capacity. In the douglas fir (Pseudotsuga menziesii (Mirb.) Franco) stands of western Oregon, the soil under 40-year-old red alder contained less than half the quantities (kg ha^{-1}) of exchangeable calcium and magnesium in the top 30.5 cm (12 inches) of the soil, compared with similar nearby soils under douglas fir and other conifers (Franklin et al. 1968). These influences of alder on the soil affect both microorganism activity and tree growth, as will be discussed later (Franklin et al. 1968, Bollen and Lu 1968, Miller 1982).

The nodulation and nitrogen-fixing activity of both legumes and non-legumes is affected by litter types and leachates from overstorey vegetation. The nodulation of leguminous black locust (Robinia

pseudo-acacia L.) and red clover (Trifolium pratensis L.) was reduced by various litter types, and goldenrod (Solidago altissima L.) litter reduced red clover nodulation by 36% to 62% (Larsen and Schwarz 1980). Rice (1968) reports that leachates of several pioneer species suppress nodulation of legumes, and suggests that this partly determines old-field succession. He also indicates that broomsedge (Andropogon virginicus L.) contains allelopathic compounds that inhibit nitrogen-fixing Rhizobium and Azotobacter. The effects of poplars (Populus) on alder nodulation was studied as part of the ENFOR-supported program at Laval University and will be discussed later in the second part of this report.

7.2 Soil animals

Soil animals play an important role in the decomposition of forest litter. They feed on soil organic matter, graze on associated organisms, mix soil and litter, inoculate litter and roots, excrete, improve aeration and, when they die, provide an organic substrate for other organisms (Hendrickson et al. 1982).

On acid soils with mor humus, primitive Collembola (springtails) which are very tolerant of cold selectively graze on fungi and modify the populations (Parkinson et al. 1979). Mites, such as Cryptostigmata, feed on bacteria and fungi, and some are predators on other soil microfauna. On the less acid mull soils large Oligochaete earthworms incorporate the newly-fallen leaves into the soil, and even in relatively acid soils small Enchytraeid worms may be active (Hendrickson et al. 1982). In films of soil water the soil nematodes and rotifers graze on soil microbes and many of these nematodes are parasitic on plant roots or prey on soil animals. The faeces from the litter-feeding soil animals elevate soil pH, mineral content, and moisture, and provide a substrate for soil bacteria (including free-living nitrogen fixers) and fungi; nitrogen fixation by free-living bacteria increases in millipede faeces (Webb 1977). As litter passes through an arthropod's gut the levels of bacteria increase more than those of fungi but, later, as the faeces decay, the fungi (Phycomycetes, Ascomycetes, and Fungi Imperfecta) increase (Nicholson et al. 1966). Soil animals speed up the initial disappearance of litter and promote humification (Hendrickson 1982).

These soil animals, therefore, interact with soil bacteria including the nitrogen-fixers, and are doubtless involved in the infection of host plants by nitrogen-fixing rhizobia and actinomycetes. The tip of a root moving through the moist soil, exuding carbohydrates and sloughing off superficial dead litter, is accompanied by a host of nematodes and other small soil fauna, bacteria, and fungi, all feeding on root exudates, root litter, superficial root tissues, and each other. Nitrogen fixation by free-living organisms and infection of root hairs by potential nitrogen-fixing and mycorrhizal symbionts, as well as soil pathogens, takes place in these conditions of intense competition for the limited nutrient and energy resources of the soil.

7.3 Leaf surfaces

Nitrogen-fixing organisms have been observed on the leaves of many tree genera in the temperate zone, such as Pseudotsuga Carr., Tsuga Carr., Larix Miller, Chamaecyparis Spach, Pinus L., Picea Dietrich, Alnus (Tourn.) L., Fagus (Tourn.) L., Quercus (Tourn.) L., and Acer (Tourn.) L. (Jones 1976). The phyllosphere associations develop on substrates of sugars and amino acids excreted by leaves. Nitrogen is in short supply on these surfaces so that when the nitrogen-fixing bacteria become established they are followed by a wider range of microorganisms. These colonies of bacteria, fungi, and algae on leaves reach their peak when the leaves are mature and actively photosynthesizing and transpiring (Jones 1976). Some of the metabolites (which may include indole-acetic-acid (IAA) and gibberellins) produced by the phyllosphere colonies can be assimilated by the plants (Ruinen 1961, Fortin et al. 1979).

7.4 Decomposing wood litter

Free-living bacteria fix nitrogen in rotting wood, but the extent of their contribution to the nitrogen cycle and breakdown of cellulose is not fully understood. Cornaby and Wade (1973) observed that microorganisms fixed about 0.9 kg ha^{-1} $year^{-1}$ of nitrogen in rotting logs of chestnut (Castanea Tourn. ex L.), and nitrogen-fixing Bacillus polymyxa, B. macerans, B. circulans, and Klebsiella pneumoniae have all been observed on rotting wood (Sharp 1975, Fortin et al. 1979). The activity of wood-rotting fungi is increased by a supply of fixed nitrogen, accelerating the decomposition of the wood fibres (Postgate 1978).

7.5 Lichens and mosses

Lichens are abundant in many forests, growing on the soil surface, rocks, tree stems, and branches. Many of them, such as Peltigera, Stereocaulon, Colleme, and Nephroma species, include nitrogen-fixing, symbiotic photo-trophs such as Nostoc sp. and Stigonema sp. (Henriksson 1971). Relatively little is known about nitrogen fixation in the moss layers of forests, although Granhall and Lindberg (1978) have recorded that as much as 37 kg ha^{-1} $year^{-1}$ of nitrogen is fixed in the sphagnum communities associated with coniferous forests.

7.6 Animals and insects

Nitrogen fixation also occurs in the forest fauna. Free-living nitrogen-fixing organisms include coliform bacteria such as the facultative Klebsiella; about 50% of the known Klebsiella species and strains are able to fix nitrogen. These bacteria resemble the coliform Escherichia coli which occurs in animal gut, and one species (E. intermedia) appears to be able to fix nitrogen. The genera Citrobacter and Enterobacter, both of which are coliforms, occur in both animal intestines and the soil. They include nitrogen-fixing strains. The bacterium Citrobacter has been observed in the guts of insects (Postgate 1978), and French (1976, 1980) observed nitrogen fixation in the hind guts of termites.

Many herbivores (e.g. ungulates) are known to have nitrogen-fixing bacteria in their rumens (Stewart 1982), but the contribution of these bacteria and those in insect guts to the forest nitrogen cycle is not known.

8.0 THE EFFECTS OF THE ENVIRONMENT ON NITROGEN FIXATION

Free-living and symbiotic nitrogen-fixing organisms, and higher plant hosts, are profoundly affected by the environment. Some of these environmental factors, such as water, pH, calcium, and nitrogen content of the soil, as well as light, can be modified by the forester. Because the response of both organisms and hosts depends upon their genetic constitution, a forester can carry out genetic selection to some extent.

8.1 Soil pH

Soil pH affects many of the soil microorganisms, nitrogen fixers and non-fixers, both directly and indirectly. Ammonia released by ammonification of raw humus may be immobilized in non-hydrolyzable forms if the pH is increased (Nommik 1970). Lowering pH can reduce nitrification, as described earlier, and modification of soil pH can affect other bacterial processes, successions, and numbers.

Most of the work on the effect of pH on nitrogen fixation has been carried out on Rhizobium symbioses in the legumes (Holding and Lowe 1971, Lie 1974). Calcium, molybdenum, and magnesium are essential to successful legume fixation, and the availability of these elements is lowered when soil pH is reduced. Not many rhizobial associations are successful on very acid soils with pHs of less than 5.1 (Holding and Lowe 1971, Lie 1974), but there is a Rhizobium strain that forms nodules on acid soils (Lie 1971). The effect of pH upon the availability of nutrients necessary for the nitrogen-fixing process, and for the growth of the host, influence both symbiotic and non-symbiotic fixation and should be understood by a forester proposing to use legumes as green crops on forest soils.

No information was located describing the response of Frankia to soil pH, but actinorhizal symbioses (Myrica, Comptonia etc.) occur on relatively acid forest soils.

8.2 Soil water

Nitrogen-fixing systems in nodules of legumes and non-legumes, as well as in blue-green algae and lichens, are sensitive to water stress (Sprent 1971). Moderate water stress in the nodules slows down nitogenase activity (as measured by acetylene reduction), nitrogen reduction, and respiration, but the effects are reversible. Severe water stress inhibits nitrogen fixation, lowers respiration, and changes the nodule structure, especially when nodules are near the soil surface, as is the case with many herbaceous legumes. There is evidence that water-logging can reduce nitrogen fixation in both legumes and non-legumes (Sprent 1971).

Many of the alders (e.g. A. rugosa) grow well on wet soils in Canada, provided there is adequate aeration, and some can tolerate drier conditions (e.g. A. crispa), so that the forester has a wide selection of material from which to choose. The legumes are not likely to be useful on wet, acid soils in Canada.

8.3 Soil cations

Temperate legumes have a fairly high calcium requirement, particularly immediately after rhizobial infection and at nodule initiation, and more calcium is needed for rhizobial nodule formation when the soil is acid than when soil pHs are higher (Lie 1974). As many forest soils in Canada have low levels of calcium, this will limit the use of legumes unless fertilizers are applied. Fortunately calcium fertilizers are relatively inexpensive to manufacture, store, and apply. No information was located about the response of Frankia to calcium.

The growth of rhizobia (e.g. R. meliloti) is inhibited by low levels of copper (Cu) when cobalt (Co) levels are low, particularly when the pH is lowered from 7.0 to 6.6. Low zinc (Zn) levels also reduce growth of rhizobia. High levels of manganese (Mn) reduce the effectiveness of rhizobia, but on the whole the rhizobia appear to be tolerant of high levels of this element (Holding and Lowe 1971). There is some evidence that different strains of rhizobia vary in their sensitivity to Cu and Mn (Sherwood 1966), but no information was lcoated about the effects of heavy metals on actinorhizal symbioses.

When a forester is considering using nitrogen-fixing symbioses in stand management it will be necessary to take into account the levels in the soil of cations such as Ca, Mg, Mn, Mo, etc. and anions (e.g. P) that affect to nitrogenase activity. Where these ions are deficient it will be necessary to modify the soil by chemical or biological means.

8.4 Soil nitrogen

Nitrogen fixation evolved as a mechanism to provide combined nitrogen in conditions where this nutrient was limiting, giving the organisms with this mechanism a distinct competitive advantage. It is not surprising, therefore, that in many situations the activity of the nitrogen-fixing system is reduced or completely inhibited by the presence of high levels of available soil nitrogen. Heavy application of nitrogen fertilizers (such as urea) generally depress nitrogen-fixing activity of both legumes and non-legume.

The formation of rhizobial symbioses in legumes is facilitated by a period of nitrogen hunger. Root nodulation is inhibited by addition of combined nitrogen, but the effect can be alleviated to some extent by applying sugar to the leaves or increasing photosynthesis. The inhibition is affected by the processes of the host, and the hypothesis that the hosts' internal C:N ratios govern nodulation nad nitrogen fixation is under examination; nitrogen fixation appears to be inhibited in plants with low C:N ratios. Nitrate (NO_3^-) inhibits the production of root hairs, which are the sites of rhizobial infection, and the growth of the rhizobial infection thread. Therefore, nitrogen levels affect infection of the hosts by the rhizobias (Lie 1974). No comparable information was located for Frankia.

8.5 Soil carbon dioxide and ethylene

Root nodules develop in contact with the soil atmosphere which contains several gases that are by-products of the activities of microorganisms and plant roots. Carbon dioxide concentrations of 3% completely inhibit nodulation of the legume Phaseolus, and other gaseous emanations from isolated roots also inhibited nodule formation, possibly due to the presence of ethylene which is known to inhibit root growth (Grobbelaar et al. 1971). In spite of nodule inhibition by 3% CO_2, the presence of some CO_2 in the root environment of legumes is necessary for optimal nodulation; the Rhizobium bacteria need low partial pressures of CO_2 for normal growth (Mulder and Van Veen 1960, Lowe and Evans 1962). The balance of CO_2 in the soil has a marked effect on nitrogen-fixation and the activities of a wide range of soil organisms, and can only be manipulated by foresters to a limited extent.

8.6 Damage to host plants

Any damage to a host plant by insects or disease will affect the nitrogen-fixing symbioses by modifying the supply of photosynthate carbohydrate. The defoliation of legumes such as clovers results in the loss of active, pink (leghaemoglobin) nodules and recovery can be slow. Acetylene reduction (nitrogenase) activity usually declines within 24 hours of defoliation and begins to increase about six days later. Herbaceous legumes in the forest may be defoliated by browsing, and arborescent legumes can be affected by insects with consequent reduction of nodulation, leghaemoglobin content, and nitrogenase activity.

8.7 Temperature

Temperature affects the growth of host plants, nodule formation, and bacterial activity at all stages. Although roots and shoots cannot be considered in isolation, the root temperatures are more important to nitrogen fixation than shoot temperatures. The optimum root temperature for nodulation of legumes such as lucerne, red clover, peas, and soybeans, is in the 20°C to 30°C range, but some strains of legumes are adapted to higher and lower temperatures (Gibson 1971). Studies of clover (Trifolium) indicate that root temperature affects the rate of nodule appearance and the appearance of leghaemoglobin which maintains low oxygen levels at the sites of nitrogenase activity (Gibson 1967).

Although there is evidence that high root temperatures can inhibit nitrogen fixation by rhizobia, the degree of inhibition varies with strains of both host plants and rhizobia. Some strains of clover rhizobia are adapted to low temperatures; strains from latitudes of 68°N to 70°N grew faster, nodulated the hosts earlier, and reduced acetylene more actively at a temperature of 10°C than strains from the south (S. of 63°N). Legumes can nodulate even in the arctic environments of Greenland and the north coast of Alaska (Porsild 1930, Allen et al. 1964, Ek-Jander and Fahraeus 1971), and it should be possible to select strains suitable for forest operations in Canada's cold climate.

Although there is some evidence that low shoot temperatures affect nodulation (Gibson 1971), the situation is less clear-cut than for root temperatures. Nevertheless, when clovers were grown with low shoot temperatures and the roots were at temperatures of 14°C and 20°C, the rate of nitrogen fixation increased considerably after increasing shoot temperatures during both the photoperiod and the dark period (Gibson 1971).

No information was obtained on the relationship between root and shoot temperature and the nodulation and nitrogen-fixing activity of actinorhizal symbioses with Frankia symbionts.

8.8 Light

Light quality and quantity affects the growth of the host plants and of nodulation. Most temperate legumes are intolerant of shade, and their use in forests will need the removal or thinning of the tree canopy. It is obvious that the effect of light on the host's photosynthesis will affect the supply of carbohydrate energy to the nitrogen-fixing loci, but there are effects of light that are not directly associated with photosynthesis.

The exposure of the roots of Phaseolus vulgaris L. to white light immediately before inoculation with rhizobia stimulates nodulation during a subsequent dark period, but prolonged exposure to strong white light after inoculation suppressed nodulation; the illumination of the roots affects formation of the symbiosis (Grobbelaar et al. 1971). There is evidence that the effect of light on nodulation may be associated with the plants' phytochrome systems, the photoreceptor system controlled by red and far-red light. Legumes (e.g. Phaseolus) grown in red light nodulated quite well, but a few minutes' exposure of roots or shoots to far-red light inhibited nodulation; under blue light there were only few, slow-growing nodules. The light in the evening is relatively rich in far-red wavelengths compared with midday and this possibly affects nodulation. Red light is absorbed by leaves of deciduous trees more than far-red wavelengths, and the inhibitory far-red light can exceed red light by eight times beneath the shade of those trees. These effects of light on nodulation will affect a forester's decisions on the use of legumes (Lie 1971). There may be Rhizobium strains resistant to far-red inhibition.

A forester considering the use of nitrogen fixation as a forestry management tool needs to understand at least the most important of these responses to environmental influences in order to use microorganisms and the associated plants effectively. Unfortunately, forest soil biology has been studied relatively little compared with agricultural soils. Thus, although much is known about rhizobial symbioses in legumes, very little is known about the autecology of the Frankia symbiont present in actinorhizal symbioses, which have the highest potential value in forest management. Neither is very much known about nitrogen fixation in the rhizosphere of forest plants, nor about the activities of nitrogen-fixing bacteria in the numerous microsites in forests. There are a many gaps in our knowledge that need to be filled.

9.0 THE USE OF NITROGEN FIXATION IN FORESTRY

Nitrogen-fixing symbioses have been used for centuries in agriculture to improve crop yield and restore soil fertility. The ancient Romans were well aware of the ability of legumes to maintain soil fertility, and in 1613 the Englishman William Brown wrote: "The alder whose fat shadow nourisheth--Each plant set neere to him long flourisheth" in one of the popular songs of the time, suggesting that it was well known that plants near alders grew well.

Although legumes have long been used in rotation agriculture and, to a lesser extent, in forest nurseries to maintain soil organic matter and nitrogen levels, the use of nitrogen-fixing symbioses never developed to any great extent in forest operations. The maintenance of soil fertility has not been high on the list of most North American forest management priorities, and fertilizers were easier to apply even though they were expensive. The rising cost of fertilizers, their unwanted effects on soil, and the intensification of forest management have led scientists to search for more benign, biologically-based soil improvement techniques.

Some host plants possessing nitrogen-fixing symbioses grow vigorously and some do not. The vigorous trees and shrubs, such as alders and Comptonia, compete with associated tree crops to an undesirable extent and foresters regard them as troublesome weeds. When nitrogen-fixing trees and shrubs are used it is necessary to design silvicultural methods that will permit the control of excessive competition by biological or chemical means. The commonly used shelterwood system is ill-suited to the use of nitrogen-fixing symbioses, while stands that are clear-cut, group-cut, or strip-cut offer opportunities for the use of rhizobial legumes and actinorhizal non-legumes.

The information in the foregoing sections of this report makes it clear that foresters will have to design new silvicltural techniques if they wish to use these symbioses. Not many of the legumes or alders tolerate a lot of shade, and forest soils, which tend to be infertile, may not possess sufficient nutrients necessary for both the nitrogenase process (phosphorus, molybenum, magnesium, etc.) and the growth of the host plants (calcium, potassium, trace elements, etc.). Nevertheless, none of these problems is insurmountable.

Some of the symbioses, such as those associated with arborescent non-legumes (e.g. alders) can make significant contributions to both fibre yield and soil fertility. Legumes will be mainly useful for improving forest soils, although arborescent legumes (Robinia, etc.) may contribute to fibre production in southern Ontario.

9.1 Effects of legumes and actinorhizal non-legumes on soil fertility and fibre yield

Legumes and actinorhizal non-legumes can improve fibre yield and soil fertility considerably. There is also evidence that actinorhizal alders reduce the risk of infection by root pathogens such as Poria and Phellinus

weirii (Nelson et al. 1978, Wallis 1968) by producing inhibitory phenols and developing specialized mycorhizal and rhizosphere populations (Tarrant and Trappe 1971). Tarrant and Trappe (1971) have described the impact of alder on the soil in their excellent review of the literature.

9.1.1 Soil improvement

There are many examples of alders improving a wide variety of soils by their litter and nitrogen fixation (Table 16) as was discussed in Section 7.0. Alnus rubra, A. glutinosa, and A. rugosa produce about 1 315 to 5 500 kg ha^{-1} of leaf litter each year, and the nitrogen content of this litter is high (2.2% to 3.5% ODW) compared with the leaf litter from associated vegetation (0.7% to 1.6% ODW). Red alder in western Canada produces about 244 kg ha^{-1} $year^{-1}$ ODW of nodules which eventually decompose and return their nitrogen to the soil. The root exudates and free-living organisms in these forest soils can contribute as much as 60% of the accumulated nitrogen, compared with 40% from the leaf litter (Zavitovski and Newton 1968). Nitrogen-rich root exudates and nitrogen fixed by free-living microorganisms in the soil enter the soil nitrogen cycle rapidly, while nitrogen in leaves takes longer to release; the foliage passes through the guts of soil animals, is broken down by fungi, and decomposed by bacteria. Little is known about the nitrogen contribution from the decay of fine roots of alders, but in some tree species (e.g. Abies amabilis (Dougl.) Forbes) fine root litter can exceed leaf litter (Grier et al. 1981), and this should be borne in mind.

In most cases alders increase nitrogen and organic matter levels of forest soils, lower pH, and modify microbial activity (e.g. nitrification). The lowered pH can result in the loss of ions of bases such as K^{+}, Ca^{++}, and Mg^{++} (Franklin et al. 1968) if exchange sites in the soil are few or weak, and soils under alders tend to have relatively high nitrate levels. More details can be obtained from Bollen and Lu (1968), Franklin et al. (1968), Tarrant (1961, 1968), Tarrant and Trappe (1971), Yamaya (1968), Miller and Murray (1978).

Alders are not the only actinorhizal non-legumes capable of improving forest soils. Ceanothus velutinus Dougl. (snowbrush) adds about 32 to 55 kg ha^{-1} $year^{-1}$ of nitrogen and increases both soil temperature and moisture in the western United States, thereby improving by 48% the stocking of logged and burned sites with Douglas fir seedlings. More research is needed on the effects of other actinorhizal symbioses (McNabb et al. 1979, Youngberg et al. 1979).

Little information about the effects of legumes on forest soils was located. The legumes, however, have been used in European forestry for some time, particularly lupins (Lupinus). Some results from experiments on poor sites have not been encouraging. Rehfuess (1979), for example, used Lupinus polyphyllus Lindl. on nutrient deficient soils under stands of Scots pine (Pinus sylvestris L.) in Germany. In order to establish lupins the site was cultivated and fertilized with both calcium and phosphorus, but the tillage caused considerable damage to the shallow roots of pines. He found that use of lupins with cultivation and fertilization increased soil calcium, phosphorus, and pH, and shifted the soil organic matter from mor to mull.

Table 16: Soils improved by alder[1]

Soil problem	Country
Erosion	Japan, Korea
Mine spoil	Denmark, Germany, United Kingdom Holland, USA
Exhausted farmland	Taiwan, Germany
Degraded forest land	Germany, Russia, USA
Raw glacial till	USA
Peatland	Ireland
Sandy soils	Czechoslovakia, Russia
Raw humus + iron pan	Germany
High water table	Poland

[1] Condensed from Tarrant and Trappe (1971).

The humus capital decreased by 7% to 33% and soil nitrogen increased by only about 15%. He concluded that use of calcium and phosphorus fertilizers with repeated doses of nitrogen fertilizer was preferable to the use of lupins on these sites. Results from this research do not necessarily imply that lupins cannot be used in forestry, because the effects of the legumes are obscured by effects of cultivation and fertilization. The soil disturbance and raising of the pH by the fertilizer would almost certainly increase nitrogen losses by ammonification and nitrification.

Experience with lupins in Russia has been quite different. Shtukin (1982) found that when lupins were established on poor soils under pine stands in Russia, and dressed with superphosphate (containing Ca and P) and potassium chloride (KCℓ), there was an increase of soil humus (18% to 20%), soil nitrogen, and yields of both pine fibre and ground vegetation browse.

It is apparent that the success of lupins in forestry depends to a large extent on the technique used, particularly the supplementary fertilization. In New Zealand soil nitrogen levels are maintained by growing lupins (Lupinus arboreus L.) on recent volcanic ash or sandy soils where Pinus radiata D. Don is grown (Silvester et al. 1979). Coriaria arborea, a non-legume, is also promising as a soil improver in that country. It is of interest to note that the main increase in soil nitrogen in Shtukin's (1982) experiment occurred when the pine canopy closed and the lupins died, thereby providing an extra nitrogen supply at a time when the leaf area index and potential assimilation of the stand were relatively high. Other herbaceous legumes have been used in forestry only to a limited extent, but Haines et al. (1978) report that clovers and vetches increased both soil nitrogen and yield in American sycamore (Platanus occidentalis L.) plantations grown on short rotations in the southeastern United States.

There is, therefore, abundant evidence that in many situations nitrogen-fixing legumes and actinorhizal non-legumes improve forest soils and have a potential role in operational forestry. Foresters planning to use these symbioses will need to bear in mind that some actinorhizal species (e.g. alders) lower soil pH and could reduce organic matter, mineralization, and nitrification. Conversely, soil disturbance by cultivation and application of calcium fertilizers which may raise the pH, could accelerate mineralization and nitrification to an undesirable extent, thereby reducing the benefits of the nitrogen-fixing symbioses. In anaerobic situations this could lead to further nitrogen loss as N_2 and N_2O from anaerobic denitrification.

Care will be necessary in short rotation poplar and sycamore systems where cultivation and fertilization are used. Both cultivation and fertilization with nitrogen, calcium, and phosphorus could accelerate nitrification and NO_3^- loss. In areas with high water tables (e.g. poplar sites), denitrification and loss of nitrogen as N_2 and N_2O would be likely, and cultivation and injudicious fertilization could lead to undesirable nitrogen and organic matter losses.

Fertilization will be necessary on many forest soils to obtain effective nitrogen fixation and growth by the symbioses, but nutrients need

to be applied with caution. The needs and interactions of the main tree crop, the microbe - higher plant symbionts, and other microorganisms involved in the nitrogen cycle will have to be borne in mind. There is a clear need to develop soil amendment techniques cognizant of the biology of both forest vegetation and soil biota.

9.1.2 Yield improvement

A good example of the effects of an actinorhizal non-legume on tree growth, is the effect of red alder on cottonwood (Populus trichocarpa Torr. and Gray). Four years after mixed stands of cottonwood and alder were established, the dry weight yields were 35% more than pure stands of either species, mainly due to the increased growth of the poplar. At the same time soil nitrogen increased (De Bell and Radwan 1979). Another example is the effect of actinorhizal autumn olive (Elaeagnus umbellata Thunb.) planted in widely-spaced walnut (Juglans nigra L.) plantations. In spite of the fact that the walnut had not responded to previous applications of nitrogen fertilizer, after nine years the height and diameter growths of the walnut associated with alder were 70% and 67% more than in pure walnut stands, respectively (Funk et al. 1979). It is of interest to note that this occurred despite the fact that walnut is well known for its strong allelopathy which could have affected nodulation of the alder.

Alders can also benefit conifers. Douglas fir was planted on a well drained, silty clay in Washington and, later, in 1953, a strip was interplanted with red alder as a fire break. After 27 years the mixed forest produced more than two times the volume of wood, had faster growing douglas fir, better stem and crown forms, and higher soil nitrogen. The stand was not designed for the experiment and there were randomization problems, but the trends were clear (Tarrant 1961, Harrington et al. 1981).

Information is sparse regarding the effects of other actinorhizal non-legumes, but Myrica appears to improve pine yields. Uemura (1971) in Japan found that mixing arborescent Myrica rubra Sieb. et Zucc. with Pinus thunbergii Parl. increased both pine yield and soil nitrogen. The control in this experiment is open to question, but the effects are indicative.

Not many data were located illustrating effects of legumes on tree growth, but Haines et al. (1978) reported that establishing clovers and vetches before canopy closure increased the growth of sycamore (Platanus) two to three times in southeastern USA. There are other examples of trees benefiting from association with nitrogen fixing symbioses (Table 17), and more information can be obtained form Haines and De Bell (1979) and Tarrant and Trappe (1971).

Many reports concerning effects of nitrogen-fixing trees, shrubs, and herbs on the fertility of forest soils and growth of trees are based on experiments that were not designed for the specific purpose, or on very small plots, or on extrapolation from laboratory or greenhouse data. There are relatively few well-designed field experiments that withstand rigorous examination. It is clear that plants with nitrogen-fixing symbioses usually

Table 17: Tree species benefiting from association with nitrogen-fixing non-legumes (after Haines and De Bell 1979, and Tarrant and Trappe 1971).

Actinorhizal non-legume genera	Sites	Tree genera benefiting
Alnus	Poor, eroded soils, sands, gravels, mine tailings, bogs	*Populus, Fraxinus Pseudotsuga Picea, Malus*
Elaeagnus	Poor soils, disturbed areas, sand dunes	*Juglans*
Myrica	Acid bogs, dunes, mine tailings	*Pinus*
Ceanothus	Dry sites, chaparral	*Pseudotsuga Pinus*
Coriaria	Sands, gravels, clays wet sites, volcanic debris	*Pinus*

benefit forest soils and frequently enhance tree growth. However, there is a need for well-designed field experiments on a variety of sites and with a variety of species, particularly in Canada, to provide a sound basis for the development of field techniques and future planning.

9.2 Silvicultural techniques

There are many ways that nitrogen-fixing legumes and non-legume symbioses can be used in forest management to increase yield and maintain soil fertility, but uses are constrained by the fact that the higher plant hosts are intolerant of a lot of shade. Consequently, smaller shrubby and herbaceous species can only be used in cleared areas (clear-cuts, strip-cuts, group-cuts) or beneath open canopies, although vigorous arborescent plants with nitrogen-fixing symbioses (e.g. alders), that are able to compete with the main tree crops, can remain within stands after the canopies have closed. Fast-growing alders can also be grown on their own to produce fibre (Fortin and Carlisle 1983).

Soil amendment may not be necessary in selection systems, but in areas where clear-cuts and slash-burning have been carried out and the site has been left unregenerated for several years, losses of soil nitrogen and organic matter are likely to be appreciable and their replacement will be slow under natural conditions. Slash-burning alone oxidizes large quantities of nitrogen and these are lost as gases. In such a situation the forester is faced with the problem of whether to leave the soil unamended hoping that time will remedy the deficiencies, or apply fertilizers, or use nitrogen-fixing symbioses (with or without fertilization). The manager's decision will be affected by costs of the operation, but he may have no option but to use the nitrogen-fixing symbiosis with or without complementary fertilization if he seriously wishes to restore both soil nitrogen and organic matter.

As mentioned earlier, nitrogen fertilizers can stimulate nitrification and trigger further losses of nitrate and later losses of N_2 and N_2O by denitrification in anaerobic microsites. If the forester spreads large quantities of organic mill wastes in forests to restore soil organic matter loss, there might be a rise in C:N ratios to undesirable levels if the nitrogen contents of the wastes are low even if the wastes are free from toxic heavy metals. Nitrogen fertilizers may give a boost to tree growth, but this does not necessarily mean that all is well with the soil in the long term. Trees grow well in hydroponic media maintained by high energy inputs, but the long term productivity of forest soils depends upon both physicochemical characteristics and the activity of the soil biota.

If the forester decides to use nitrogen-fixing legumes and non-legumes he may need to select a fertilization régime to encourage development and activity of these symbioses, and modify his herbicide treatment to take into account effects of the phytotoxins on soil organisms. It may also be necessary for him to ensure that suitable mycorrhizal symbionts develop on the nitrogen-fixing system to promote phosphorus uptake (for ATP energy) in order to minimize the need for phosphate fertilizer. The forester will need to develop a holistic approach taking into account the needs and responses

of both the trees and the soil biota, but relatively few foresters are sufficiently well informed about soil biology to take full advantage of this approach (Fortin and Carlisle 1983).

Although plants with nitrogen-fixing symbioses can be used in the management of "natural" forests, their greatest potential is in plantation forestry, particularly in the intensive silvicultural systems that involve short rotations, whole-tree harvesting, and fast-growing tree genotypes. These highly intensive systems hold great promise for the production of wood fibre for energy and other products that do not require trees of large dimension. Nevertheless, the more intensive the silvicultural system and the more it differs from complex "natural" forest, the more energy will be required to maintain the system and site productivity. Foresters will need to bear in mind the relatively high energy inputs required in intensive silvicultural systems, and to minimize the use of energy from non-renewable resources. Nitrogen-fixing plants are the most promising available tools, because they produce combined nitrogen by using solar energy. Although fertilizers may be needed to establish the symbioses, the chemicals will be mainly bases such as calcium and magnesium and micronutrients such as molybdenum, and not the costly, energy-demanding nitrogen fertilizers.

The choice of silvicultural system involving plants with nitrogen-fixing symbioses will depend upon management objectives. For the purposes of this report it will be assumed that the goal is high fibre production and maintenance of site productivity. If the forester wishes to provide wildlife browse his choice of nitrogen-fixing plants will be constrained by their palatability, and where secondary products are required, it may be possible to use lupin species with seeds of high oil content.

Haines and De Bell (1979) have discussed the cultural systems available to foresters wishing to use plants with nitrogen-fixing symbioses (Table 18). They suggest that plants with nitrogen-fixing symbioses can be grown on rotation between crops of trees unable to form symbioses. The nitrogen-fixing plants may be simply a green manure (e.g. legumes) to be killed with herbicides and ploughed in, or may be a commercial species such as red alder. This approach avoids the problem of growing plants with nitrogen-fixing symbioses beneath tree canopies. The green-manuring system is well suited to short rotation systems on relativley fertile soils (e.g. poplar, sycamore, etc.), but has the disadvantage that it takes land out of the fibre-producing cycle for 1 to 3 years.

The mixed species system described in Table 18 has the advantage that land is not taken out of the fibre-producing cycle. The disadvantage, however, is that the nitrogen-fixing plant may compete excessively, need control, and not survive long enough to be of extended benefit if the main non-fixing tree crop grows rapidly and quickly closes the canopy, shading out the intolerant nitrogen-fixing plants. These problems can be overcome by use of suitable species and spacing, but techniques need development.

Nitrogen-fixing trees can also be used as the principal crop if they produce commercial fibre. In most of Canada only alders will be suitable

Table 18: Different cropping systems using plants with nitrogen-fixing symbioses (after Haines and De Bell 1979)

1. Rotation systems
   a. Green-manuring with nitrogen–fixing plants that do not produce commercial fibre, followed by a commercial tree crop[1].
   b. Alternative crops of commercial tree species, one able to form nitrogen–fixing symbioses and the other unable to do so.
2. Mixed species systems
   a. Mixtures of two commercial tree species both able to form nitrogen–fixing symbioses.
   b. Nitrogen–fixing species with no commercial fibre value beneath the overstorey of a non-fixing commercial tree species for most of the rotation.
   c. Nitrogen–fixing species with no commercial fibre value beneath an overstorey of a non-fixing commercial tree species only until the canopy closes.
3. Nitrogen–fixing trees as the principal crop

[1] Such a system can produce a commercial product from herbaceous legumes (e.g. oil seed from lupins).

for this approach, but in southern areas it may be possible to use Robinia and in relatively mild coastal areas there will be a wider range of options. The forester will, of course, need to use nitrogen-fixing plants suitable for the sites concerned. On poorer soils non-legumes such as Alnus, Myrica, and Comptonia could be suitable, but when considering Comptonia and Myrica it will be necessary to bear in mind that they are telial hosts to the sweet-fern blister rust (Cronartium comptonia Arth.) and should not be used near trees sensitive to that fungus, i.e. Pinus banksiana Lamb., P. ponderosa Laws. and P. contorta Dougl. (Davidson and Prentice 1967).

9.3 Genetic improvement of alder

The alders of potential value in Canadian forestry, Alnus rubra, A. crispa, A. rugosa, A. glutinosa, and A. incana, all have extremely wide distributions in their native habitats. Even the relatively sensitive Alnus rubra, with its mainly coastal distributions in the western North America, extends from latitudes of about 34°N to 60°N (Johnson 1968). While these large and variable populations provide vast pools of genetic material of great potential value, it also means that foresters will need to take particular care in their selection of alder provenances.

Although A. rugosa and A. crispa are very hardy and vigorous, they are mainly shrubby and not well suited to fibre production. Within their vast populations, however, it is likely that there are arborescent strains with well defined main stems and apical dominance, and an effort is necessary to locate, select, and propagate them. It is known that some alders hybridize (Hall and Maynard 1979), so it may be possible to create hybrids of the hardy, vigorous, shrubby types and the arborescent types to combine the stem form, hardiness, and vigour.

Many alders have the advantage of being very close to a forest geneticist's "ideotype" (ideal tree). Alders have wide distributions, great variability (which includes some genotypes with desirable stem and crown forms, hardiness, and rapid growth), early flowering, and the ability to coppice well. They also form nitrogen-fixing symbioses with Frankia, a characteristic that appears to be genetically variable. Some of alders' weaker points are their demands for relatively high soil moisture, a photosynthetic efficiency that does not appear to be particularly high, and wood properties that are not at present regarded as very desirable by some sectors of the forest industry. Nevertheless, all these less attractive features are genetically variable and could be genetically manipulated (Gordon 1978).

Little work has been carried out on the genetics of alders in North America, and it is an open field for Canadian forest scientists. The arborescent red alder (A. rubra) has been studied in the USA and there appears to be considerable within-provenance variation. De Bell and Wilson (1978) found that, in an 8-year-old Alnus rubra provenance near Olympia, Wash. involving genotypes from Alaska, British Columbia, Washington, Oregon, and Idaho, the fastest growing trees came from northwestern Washington. Trees from British Columbia, southwestern Washington, and Oregon also

performed well, and survival rates ranged from 97% to 100%. At 8 years-of-age, the mean diameter of trees in the best provenance was about 9.4 cm (3.7 inches) and the height about 9 m (30 feet), while provenances from Juneau, Alaska, and Sandpoint, Idaho, grew much more slowly, but were more resistant to frost. The tree characteristics studied included stem lean, stem form, crown width, branch angle, branch diameter, wood density, and bark thickness. The only characteristics that varied significantly between provenances were crown width, branching, and bark thickness, but there was considerable tree-to-tree variation in all traits within the provenances. The authors concluded that individual tree selection over large breeding zones could be an useful approach in red alder selection and improvement.

Alders hybridize in the natural state and many artificial hybrids (Table 19) have been created (Murai 1964, Hall and Maynard 1979). The hybrid Alnus rubra x A. glutinosa grows well (Ljunger 1959), suggesting that the arborescent habit and rapid growth characteristics of red alder might be transferred to more cold-resistant species. There is, however, evidence of hybridization barriers (e.g. retarded pollen-tube growth) in some cases (Millar 1977, Hagman 1970, 1975). Within the subgenera Alnus used in Furlow's (1974) and Chiba's (1966) classifications hybridization is common, but no crosses have been reported within the subgenus Clethropsis. Crosses within the subgenus Alnobetula (e.g. A. firma x A. sieboidiana, A. firma x A. pendula) can be achieved, but growth performance is not known (Hall and Maynard 1979).

Although the chromosome number of most alder species is 28, some Japanese alders (Alnus sieboldiana, A. firma, and A. inokumae) have 56, 42, and 14 chromosomes respectivley. Occasional individuals within species normally possessing 28 chromosomes have 42 or 56 polyploids (Table 20), and the existence of polyploidy in the genus Alnus may provide breeding opportunities (Murai 1964, Chiba 1962, Stettler 1978, Gram et al. 1941, Johnsson 1951). Polyploidy in the genus Alnus is confusing, since there is uncertainty about the basic chromosome number. Scientists tend to regard the "normal" chromosome number as diploid (2n = 28), with a haploid number of n = 14, triploids of 3n = 42, and tetraploids of 4n = 56 (Table 20). Chiba (1966), however, makes a good case that the basic number is 7 (similar to related Betula), implying the "normal" alders with 28 chromosomes could be tetraploids and alders with 56 crhomosomes may be octoploids (Gram et al. 1941). The position is still far from clear (Hall and Maynard 1979).

The growth of triploids in some tree species (e.g. Betula, Malus) is often good, but the performance of tetraploids has generally been disappointing (pers. obs.). Many of the "tetraploid" (n = 28) alders grow very well which, if they are truly tetraploid, is a little surprising.

The variability of alders has led Stettler (1978) to conclude that red alder offers plenty of material for genetic improvement, suggesting that hybrids of A. rubra, A. glutinosa, A. rhombifolia, and A. incana have promise. He also suggested that the red alder for use in short rotation forests should be selected for rapid juvenile growth, good fibre properties,

Table 19: Successful hybridization of alders (*Alnus*)[1]

Hybrid		Reference
A. glutinosa[2]	x *A. hirsuta*	Hall and Maynard 1979
A. glutinosa	x *A. inokumae*	"
A. glutinosa	x *A. orientalis*	"
A. japonica	x *A. glutinosa*	"
A. cordata	x *A. glutinosa*	"
A. rubra	x *A. glutinosa*	"
A. incana	x *A. glutinosa*	"
A. incana	x *A. subcordata*	"
A. incana	x *A. japonica*	"
A. incana	x *A. orientalis*	"
A. incana	x *A. hirsuta*	"
A. japonica	x *A. inokumae*	"
A. japonica	x *A. subcordata*	
A. rubra	x *A. glutinosa*	Ljunger 1959

[1] Authorities for species names not given by the authors.
[2] Female parent listed first.

Table 20: Polyploidy in *Alnus*

Species	Degree of Polyploidy[1] [3]						
	2x	3x	4x	5x	6x	7x	8x
	(No. of chromosomes)						
A. inokumae	14	28					
A. hirsuta		28					56
A. glutinosa		28			42		56
A. subcordata[2]		(28?)			42		56
A. orientalis[2]		(28?)			42		(56?)
A. japonica		(28?)			42		56
A. rubra x *glutinosa*					42		

[1] Assumes n = 7 = haploid.
[2] + putative hybrids.
[3] Hall and Maynard 1979, Gram *et al.* 1941, Johnson 1951, Chiba 1966.

good sprouting after mechanical coppicing, and nitrogen production. If log production is the goal he proposed that sustained volume growth, good stem form and branching, moderate seed production, and good fibre quality should be selected. He suggested using either mass selection from the best trees left to produce seed, or tests of progeny from plus-trees with grafted clonal seed orchards of the best material. Hall and Maynard (1979) give other alder improvement strategies.

No detailed information was located about the genetics and improvement of actinorhizal tree and shrub species other than alder. The genetics and breeding of legumes is not within the scope of this report.

9.4 Propagation

Alders reproduce by seed and coppice sprouts in the forest, and can be propagated by cuttings and tissue culture in the greenhouse and laboratory. There do not appear to be major propagation problems.

Red alder produces seed at about 10 years-of-age and the optimum seed production occurs at about age 25 years, with good seed years every four years or so and moderate seed crops in between. "Cones" can be opened by air-drying for several weeks or kiln drying at 26°C to 37°C (80°F to 100°F), and a two bushel sack of red alder cones yields about 0.7 kg (1.5 lbs) of 95% filled seeds after they have been cleaned by screening and blowing. Whether or not the seeds benefit from moist-cold stratification is a point in question. About 60% unstratified red alder seed germinated without stratification at a trial in Duncan, B.C., while 67% of the stratified seed germinated. Storage for one year at -19°C reduced germination of unstratified seed to 50%. Soaking for 24 hours after 3 years storage gave the best germination (c. 30%) in a trial at the University of Washington. Red alder seed has phytochrome-controlled germination, i.e. red light promotes germination and far-red light inhibits germination. The seedlings appear to grow well in containers with a 1:1:1 peat-vermiculite-perlite substrate, or can be easily raised as bare-root stock (Kennedy 1978).

Alder cuttings have been rooted, but success has been variable (Zach 1941, Kenady 1978, Schmid 1972). It is possible to get 50% to 100% success with greenwood cuttings of red alder by using indole-3-butyric acid (IBA) together with mist. Monaco et al. (1978) had great success (Table 21) rooting red alder cuttings using 4 000 to 8 000 ppm IBA + 10% benomyl fungicide with high humidity (by mist or plastic bags), a 16-hour photoperiod, 4 400 to 5 000 lux light, day temperatures of 22° to 25°C, and night temperature of 16° to 22°C. Other alder species can be rooted in Canada in the summer by using green cuttings in mist, but older woody cuttings do not readily root (Zdrazil Z., pers. comm.).

Information about techniques of grafting alders is sparse. Lepisto (cited in Kenady, 1978) found that scions from mature alders can be grafted near the tips of 1 to 2-year-old root-stocks using side veneer grafts. Reliable grafting techniques need to be developed for alders.

Table 21: Rooting of cuttings of red alder (*Alnus rubra* Bong) (abstracted from Monaco *et al.* 1978)

Condition[1]	Treatment	Rooting %
A	4 000 ppm IBA[2] + 10% benomyl	78
A	8 000 ppm IBA + 10% benomyl	82
A	10 000 ppm IBA + 10% benomyl	50
B	4 000 ppm IBA + 10% benomyl	100
B	8 000 ppm IBA + 10% benomyl	100

[1] Condition A = Temp. 22°/16°C., Bottom heat 25°C., Overhead mist, 16 hr. photoperiod., 4400 lux
Condition B = Temp. 25°C/22°C., Poly bags, 16 hr. photoperiod, 5000 lux
[2] IBA = indole – 3 – butyric acid.

It is possible to propagate alders in tissue culture. *In vitro* culture of alder (*A. glutinosa*) was carried out as early as the 1930s, but interest waned until the 1970s. Since then *A. rubra* has been cultured *in vitro* from the shoot tips, axillary buds, and hypocotyls of seedings, and *A. glutinosa* was cultured from seedling shoot tips. Other nitrogen-fixing trees and shrubs such as *Gleditsia tricanthus* var. *inermis* Willd., *Paulownia taiwaniana*, *P. tomentosa* Steud., and *Robinia pseudoacacia* L., have also been successfully cultured from shoot tips, axillary buds, petioles, leaf bases, and ovule callus (Brown 1981). Recent work at Laval University, Quebec, indicates that *Alnus*, *Shepherdia*, and *Elaeagnus* can all be grown in tissue culture (Lalonde, pers. comm.).

The alders appear to be promising candidates for genetic manipulation both by conventional selection and breeding and by somatic fusion, haploid breeding, and induced ploidy.

9.5 Herbicides and fertilizers

A forester will probably find it necessary to use herbicides and fertilizers to some extent when incorporating plants with nitrogen-fixing symbioses in intensive silvicultural systems. Although the main purpose of using nitrogen-fixing symbioses is to maintain soil nitrogen and organic matter levels and minimize use of costly nitrogen fertilizers, these symbioses should not be regarded as absolute substitutes for fertilization (Fortin and Carlisle 1983).

Herbicides may also be needed to control weed growth and to kill nitrogen-fixing plants when they have served their purpose or compete excessively. Fertilizers and herbicides affect both free-living and symbiotic micro-organisms in the soil as well as the host plants.

9.5.1 Fertilizers

The effects of fertilization on nitrogen fixation *per se* were discussed earlier in this report. To recapitulate, nitrogen fertilizers affect nodulation, nitrogen-fixation rates, and nitrification, all of which are sensitive to nitrogen levels and changes in soil pH caused by fertilizer ions. Calcium, magnesium, phosphorus, and molybdenum are all necessary for the nitrogen fixation process. The calcium fertilizers will increase pH on many soils, and thereby affect both nitrification and nitrogen fixation. Any increase in nitrification due to elevated soil pH could lead to increased denitrification if anaerobic sites are available. If a forester plans to use legumes on relatively poor soils low in bases it would be advisable to consider applying calcium, phosphorus, and molybdenum.

Fertilizers such as superphosphates will supply calcium and phosphorus, but costly phosphorus supplements may not be necessary if there are phosphate minerals in the soil and well-developed mycorrhizae on roots of plants with nitrogen-fixing symbioses. In such a situation it may be preferable (and more economical) to lime or apply dolomite (Ca + Mg) bearing in mind the likely elevation of soil pH and its effect on nitrification. The increased pH will also increase molybdenum availability if molybdate minerals are present (Fortin and Carlisle 1983).

Molybdenum, which is involved in electron transport, occurs as the molybdate anion (MoO_4^-) in soils, or as the $HMoO_4^-$ ion when conditions are acid, higher pHs favouring molybdenum availability. Molybdenum can be applied as ammonium molybdate ($(NH_4)_2\ MoO_4$), anhydrous sodium molybdate (Na_2MoO_4), and molybdic oxide (MoO_3); these compounds contain 49%, 46% and 58% to 62% molybdenum respectively, and can be applied as the raw chemical or as silica frits (Burgess 1983). Ideally, a molybdenum compound should be incorporated into a slow-release Ca + Mg + P fertilizer for use with legumes, but it is not known whether such a product exists.

The same principles apply when planning to use actinorhizal symbioses such as Alnus, Myrica, and Comptonia; it will be necessary to apply calcium, magnesium, phosphorus, and molybdenum if these elements are markedly deficient. Unfortunately, so little is known about the autecology of actinorhizal symbioses and the actinomycete symbionts (Frankia spp.) that it is not possible to be more specific (Fortin and Carlisle 1983).

Addition of suitable fertilizers will, of course, affect free-living nitrogen-fixing microorganims in the soil as well as associated bacterial processes of organic matter mineralization, nitrification, and denitrification. The effects upon the rhizosphere, where it is suspected that nitrogen-fixation may be more significant than is generally appreciated, could be particularly important. However, little is known about these effects.

In the past managers have designed their soil amendment programs just to meet the immediate requirements of host trees, but if nitrogen-fixation is to be used as a management tool it will be necessary to amend soils to meet the needs of the main tree crop, the host tree, and the microbial symbiont. As Bengsten (1979) wrote, referring to the natural processes of nutrient accretion and cycling, we need "...highly selective use of fertilizers as catalysts for these processes rather than massive substitutes for or short cuts to them."

9.5.2 Herbicides

Herbicides are widely used in both agriculture and forestry to control weeds and reduce undesirable competition. Most foliar herbicides eventually reach the soil in the following ways: direclty, by leaching, through root exudates, and the decay of the dead vegetation. These toxic chemicals have been the cause of widespread public concern due to their potential effects on public health.

Agriculturalists have long been concerned about the possible effects of these toxins on soil organisms such as Nitrosomonas, Nitrobacter, and the diazotrophs that play vital roles in soil nitrogen processes. But foresters, who do not appear to be so aware of the importance of soil biota, have expressed little or no concern. Managers have tended to be reassured by the fact that many herbicides degrade rapidly in the soil. Although the products of degradation are usually less toxic than the original herbicide, they have different biological effects and can persist for long periods (Greaves et al. 1976).

Even in agriculture there is no clear-cut answer to the question of whether herbicides affect soil organisms in a way that reduces soil fertility, because techniques of assessing the role of microbiota in maintaining soil fertility have not always been reliable, and the processes are only partially understood. Modern techniques should shed more light on the role of soil organisms in soil fertility and site productivity (Greaves et al. 1976).

As far as the nitrogen-cycle is concerned, the bacterium Nitrosomonas (which converts ammonium to nitrite in the first stage of nitrification) is more sensitive to herbicides than Nitrobacter (which completes nitrification by convering nitrite to nitrate) (Greaves et al. 1976). The application of herbicides can therefore result in a build-up of ammonium nitrogen. Whether this is harmful or beneficial depends upon the tree species involved because trees vary in their needs for ammonium and nitrate ions (Van Den Driessche 1971). The less mobile ammonium ions are, however, less likely to be lost by leaching than mobile nitrate ions which can be further reduced by denitrification to mobile nitrogen gas and nitrous oxide in anaerobic soil microniches.

Some herbicides inhibit photosynthesis and affect both green soil algae, and blue-green nitrogen-fixing phototrophs (Venkataraman and Rajyalakshmi 1972, Da Silva et al. 1973), but the latter appear to recover fairly quickly from the effects. The phytotoxic activity of toxins increased at higher herbicides concentrations and with lowered soil pH (Kirkwood and Fletcher 1970, Fletcher et al. 1970).

The effect of herbicides on free-living, nitrogen-fixing bacteria varies with the type of chemical, the plants involved, and the strain of the organisms. Populations of nitrogen-fixing Azotobacter in the rhizospheres of agricultural crops such as maize and potatoes were reduced by 2,4-D herbicide (Bagyaraj and Rangaswami 1972, Tyagni-Ryadno 1969), while the same herbicide stimulated nitrogen-fixing Clostridium in the rhizospheres of the same plants. No information about the effects of herbicides on nitrogen-fixing bacteria in rhizospheres of forest plants was located.

The rhizobial symbioses of legumes are affected both positively and negatively by herbicides such as simazine. Simazine dosages of 8 ppm had little effect on the symbiosis, but higher dosages inhibited 12 out of 14 Rhizobium strains on the legume Phaseolus, thereby reducing nodule formation; simazine also reduces nodulation and nitrogen fixation in other crops such as alfalfa, clover, and peas. There are indications that host plants (e.g. clovers) are more affected by herbicides than the Rhizobium, and that herbicide-bacterium interactions vary a great deal (Greaves et al. 1976). There is no information about the effects of herbicides on the Frankia symbiont of actinorhizal trees, such as alder.

Mycorrhizae are also influenced by herbicides and this affects the ability of mycorrhizal fungi to increase the uptake of phosphorus necessary for effective nitrogen-fixation by the enzyme nitrogenase. Triazine herbicides affect both the morphology and population structure of

ectomycorrhizae, and simazine can increase the number of mycorrhiza on pines (Smith 1973). High concentrations of simazine and atrazine tend to stimulate some species of ectomycorrhizae, while other herbicides such as 2,4,5-T, dichlorobenil, and chlorthiamid inhibit growth of actomycorrhizae (Greaves et al. 1976). Phosphorus uptake by Scots pine (Pinus sylvestris L.) via the ectomycorrhizae can be inhibited by a trace (1 ppm) of 2,4,5-T, but only slightly (10%) inhibited by 120 ppm of chlorthiamid. No information was located about the effects of herbicides on endomycorrhizae.

Although it is clear that herbicides affect free-living nitrogen-fixing soil bacteria, nitrogen-fixing symbioses, and other bacteria involved in the nitrogen fixation, these interactions are far from consistent. As Greaves et al. (1976) said, "Contradictory results are the rule rather than the exception," but these effects should not be ignored. The wide gaps in our knowledge about the effects of herbicides and other chemicals on nitrogen-fixing symbioses in higher forest plants need to be filled.

The vigour of some alders makes control necessary when planted in mixtures. Newton (1978) found that injections of trichlorpyr, 2,4-D amine, and cacodylic acid can be used to thin young alder stands in the western United States, and that alder and associated species can be controlled by applying 2,4,5-T before bud burst. Alder, hazel (Croylus), thimbleberry (Rubus parviflorus Nutt.) and cherry (Prunus) can be controlled by applying 2,4-D + 2,4,5-T in late summer. The costly "brown-and-burn" method has been used in the western United States to control alder and other brush, in which the brush is slashed in the summer, and then treated with a systemic herbicide. A desiccant such as dinoseb is applied to any remaining green vegetation in late July or August; about ten days thereafter the site is burned.

It should be noted that these trials refer to the United States and the use of some of these herbicides may not be permitted in Canada. Alder can be controlled with a range of herbicides provided care is taken about dosages, timing, site, and weather.

9.6 Culture of and inoculation by nitrogen-fixing symbionts

A forester considering using plants with nitrogen-fixing symbioses has the option of leaving inoculation to chance in the containers and transplant lines, or using stock that has been deliberately inoculated with selected nitrogen-fixing microorganisms. In most soils there are free-living Rhizobium and Frankia species and plants are likely to nodulate, possibly after a short delay. Unfortunately, both Rhizobium and Frankia are very variable organisms and it is possible that inefficient wild symbionts would become established. The same applies to mycorrhizae which can contribute phosphorus to the nitrogen-fixing symbioses. If superior microbial and fungal strains are available it is preferable to inoculate the seed or plants prior to establishment in field conditions.

In the case of Rhizobium there are many strains available at agricultural centres throughout the world. There is a bank of Frankia strains at Laval University, Ste.-Foy, Quebec and this will be described in

Part II of this report. There are also banks of mycorrhizae available at many universities and government research centres.

The technique of selecting, culturing, storing, and inoculating Frankia strains was part of the work supported by the ENFOR contracts and will be discussed in Part II. Ways of handling endo- and ectomycorrhizae have been summarized by Fortin and Carlisle (1983); this report is concerned only with handling the Rhizobium symbiont.

Agriculturalists developed methods of culturing Rhizobium and inoculating legumes with the nitrogen-fixing microbe many years ago. Vincent (1970) reviewed the techniques as part of the International Biological Program (IBP), and the following is based on his report:

Rhizobium strains can be recovered from either fresh or cold-stored legume nodules if care is taken to avoid contamination. The bacteria are cultured in aseptic liquid media containing yeast extract and mannitol or an equivalent compound, and can be maintained in the dried or freeze-dried state or on agar slopes.

Legumes can be inoculated with Rhizobium strains by applying bacteria to seeds before sowing. The liquid or agar cultures are mixed with soil and peat, and suspended in water with 40% neutral gum arabic as an adhesive. The seeds are mixed with the slurry and coated with calcium carbonate to protect the inoculum, and sown immediately after pelleting. Prolonged storage of the coated seeds is undesirable.

The persistence of selected microbial symbionts in the soil is a subject not fully understood. In the case of legumes, the Rhizobium-infected nodules are short-lived, particularly when the legume is an annual. If the nitrogen-fixing activity of the selected Rhizobium in the nodules is inhibited for any reason, the superior strains is likely to be replaced by less efficient wild Rhizobia. After the legume dies back, the nodules decay and release the rhizobia into the soil, where they may or may not survive in competition with free-living microorganisms (Fortin and Carlisle 1983).

This situation is different from the perennial actinorhizal trees and shrubs such as alder and Myrica with their Frankia symbionts. Although little is known about the persistence of selected Frankia, the fact that many of the actinorhizal nodules are perennial, and even 'woody', makes it likely that the selected endophyte will persist for a number of years, giving young plants the boost they need after planting (Fortin and Carlisle 1983). Investigating the persistence of selected Frankia inoculants in the field is part of the ENFOR-supported research at Laval University.

9.7 Pests and diseases of alders

Alders are subject to attacks by a number of insects and diseases, and some of the pests can do a great deal of damage. The forest tent caterpillar (Malacosoma disstria Hbn.) and the western tent caterpillar (Malacosoma californicum Packard.) are both serious lepidopteran defoliators (Sippell

and Ewan 1967) that attack a range of hardwood species including alder (Gara and Jaeck 1978), and severe attacks are common in both eastern and western North America (Condrashoff 1957, Hildahl and Reeks 1960, Gara and Jaeck 1978). Red alder is a preferred host for M. californicum. Outbreaks of these caterpillars occur about every eight years, each attack reducing tree growth for about two years by as much as 86% (Kulman 1971, Wellington 1957). Forest tent caterpillars have been effectively controlled in Canada by viruses, pathogenic fungi, and bacteria (Morris 1969). The insecticides bidrin and phosphamidon have been used successfully to control the insects in the United States (Oliver 1964). Alders can also be defoliated by both the adults and larvae of flea beetles such as the chrysomelid Altica ambiens (Lec.) in as little as four weeks (Keen 1952).

Sawflies cause serious alder defoliation in both eastern Canada and British Columbia (Borden and Dean 1971). The introduced red-backed sawfly, Eriocampa avata L., can breed two generations in one year, and could become a serious pest of alder because it seems to resist biological control. The introduced striped alder sawfly (Hemichroa crocea (Fourcroy)) also has two generations a year and could become a serious defoliator (Gara and Jaeck 1978).

The alder bark beetle (Alinphagus aspericollis (Lec.)) could become a threat to red alder stands if increased harvesting of that species results in a build-up of logging slash within which the beetle populations can develop. In order to avoid future damage by this beetle it will be necessary to minimize slash in forest operations. The three ambrosia beetles Gnathotrichus, Trypodendron, and Zyleborus, which feed on felled logs and slash, could reduce the value of the logs by staining and bore holes, but do not pose a threat to the living trees (Gara and Jaeck 1978).

These pests will need to be considered in alder management. The forest tent caterpillars and bark beetles can be controlled either biologically, with insecticides, or by good stand hygiene during logging. The most serious insect pests in the future are likely to be the imported defoliating sawflies.

Few data were located concerning the diseases of alders that have management implications. Alders tend to become decadent at the age of 40 years (Worthington 1957) but this is not necessarily due to pathogens. About 167 species of fungi occur on red alder on sites in northwestern America and Canada including a canker disease, a flower disease (catkin broom), leaf spot diseases, powdery mildews, and a white trunk rot. Trunk rot by Fomes igniarius has caused losses of merchantable alder wood, and Fomes annosus (Fr.) Cke. also has been reported on Alnus (Shaw 1973). Hypoxylon fuscum Pers. ex Fr. causes a white-type rot on dead and dying alders, but appears to be a weak parasite (Driver 1978, Rogers 1968). Alder diseases were reviewed as part of the ENFOR-supported work at Laval University.

Alders appear to be resistant to the root disease Poria weirii Murr. (Wallis 1968) due to phenolic and other exudates described earlier. There is some evidence that in mixed stands of red alder and douglas fir the presence of the alder and its effects on soil nitrate, pH, phenols, and soil

microorganisms helps to protect the douglas fir from damage by this root pathogen (Nelson et al. 1978).

9.8 Utilization of alders

Arborescent alders have the advantage that, in addition to increasng soil nitrogen and organic matter, they produce wood fibre that can be used for energy, lumber, or pulp. Smith (1978) found that the high yields of young (8 to 10 years) red alder stands (26 to 30 O.D. tonnes ha^{-1} $year^{-1}$) and the relatively high calorific value (4.7 K cal gm^{-1} O.D.) of the fibre and bark make this species a candidate for energy plantations, but its usefulness is limited by the availability of suitable land.

There is no information about the potential use of fibre from other alder species in Canada, but it is anticipated that, although the calorific value of other alders are presumably similar to those of red alder, the yields are not likely to be so spectacular. Nevertheless, good stem form is irrelevant when trees are destined to be used for energy, and vigorous shrubby alders could well make a contribution to energy supplies.

Red alder produces trees of lumber dimensions (e.g. 30 cm dbh at 40 years) and has been used for furniture, cabinets, paneling, and pallets. The wood has a fine uniform grain, smooth texture, a pleasing light yellow-brown color, the ability to hold nails and accept paints, and little tendency to split, but in the green state alder logs rot rapidly and need kiln drying soon after cutting (Atterbury 1978).

Although red alder pulp has lower strength properties than soft wood pulp, it can be blended with the latter. The alder can be pulped by kraft or sulfite processes and the chemical pulp, which is smooth and soft with good printability, has properties similar to birch pulp. Straight red alder logs are easy to debark soon after harvest, but the logs are frequently crooked and prevent debarking problems. Alder chips need to be used soon after chipping because they color rapidly and lose strength in storage. In spite of these problems red alder is a useful pulpwood species in the west, and appears to be well suited to whole-tree chipping (Hruitfiord 1978).

Red alder is the only alder that has been used in industry to any extent in Canada and the United States. More information on the growth, energy content, and pulp properties of other arborescent and shrubby alders is needed so that full advantage can be taken of their fibre wherever their use to improve soil and tree growth is contemplated.

9.9 The costs and benefits of using biological nitrogen fixation in forestry

A forester considering using nitrogen-fixing symbioses will base his decisions upon a comparison of the investment alternatives available to him. There is ample evidence that the application of nitrogen fertilizer, either alone or mixed with other fertilizers, results in an increase in growth of the main tree crop in Canada, the United States, and elsewhere (Burgess 1983). For example, the application of urea to Picea mariana on glacial

till in Quebec, to Pinus banksiana on silty loam in Ontario, and to Pseudotsuga menziesii on a gravelly, sandy loam in British Columbia increased the growth of all these species (Weetman et al. 1980, Morrison and Foster 1977, Otchere-Boateng and Ballard 1978). There are many examples of increased yields due to other fertilizers on forest soils in the United States (e.g. Wittwer et al. 1980, Ward and Bowersox 1970, Van Cleve and Moore 1978, Shoulders and Tiarks 1980, Burgess 1983), but where several fertilizers have been applied, it is often difficult to decide the real cause of the growth response.

Some of the responses to fertilizers have been spectacular. In New Zealand, for example, Mead and Gadgil (1978) found that when 224 kg ha^{-1} of phosphorus fertilizer were applied to 20-year-old Pinus radiata on soils deficient in phosphorus, growth increased over a period of 24 years by 775 m^3 ha^{-1}. The response of the same species on a marginal site to an application of 200 kg ha^{-1} of nitrogen fertilizer at 8 to 16 years of age was of shorter duration (8 years) and smaller magnitude (40 to 60 m^3 ha^{-1}). Responses to nitrogen fertilizer alone have been relatively modest in North America, e.g. increases of 14 m^3 ha^{-1} over 5 years for Pinus taeda and 14 to 56 m^3 ha^{-1} over 7 years for Pseudotsuga menziesii (Haines et al. 1976, Miller and Fight 1979, Ballard 1979). When nitrogen is combined with phosphorus, the growth increases are sometimes slightly greater (Ballard 1979, Pritchett and Gooding 1975).

A forester will have these or similar results in mind when deciding whether or not to supplement conventional fertilization with nitrogen fixing methods, and he will need to anticipate the costs and availability of fertilizers. Theoretically, nitrogen fertilizer should become scarce and expensive simply because energy costs will tend to increase in the long term, and the manufacture of nitrogen fertilizers involves energy-consuming industrial nitrogen fixation, quite apart from energy used in transport and application. Increasing demand for fertilizer by the agricultural sector will also place upward pressure on prices.

Current industrial nitrogen fertilizer technology is based on the use of natural gas (a nonrenewable resource) for both process energy and the hydrogen gas reductant (Rottink et al. 1979). Natural gas supplies appear to be abundant in the short term, ammonia prices have fallen considerably (Douglas 1978), and there are indications that urea prices will increase at the rate of inflation. Some authorities (e.g. Douglas 1978) feel that nitrogen fertilizer costs are at present unrealistically low and barely cover the cost of natural gas. In the short term, therefore, the availability and pricing of combined nitrogen looks fairly promising (Rottink et al. 1979), but the long term picture is less bright.

Nitrogen fertilizers have the disadvantage that they can be lost either as gases or leachates. Applications of urea in the Pacific Northwest of the United States have involved lossed of 10% to 25% of the applied nitrogen as ammonia following ureolysis (Crane 1972), and about 50% of the nitrogen applied to slash pine as ammonium sulphate was lost (Mead and Pritchett 1975). Nevertheless, Miller and Fight (1979) report that the application of forest energy results in more usable wood energy than was originally invested.

Nitrogen fertilization has the advantage that, at least superficially, it is uncomplicated and easy to control. However, economic analyses of fertilizer use in forestry have given variable results. Some fertilization is economic and some not. Beuter (1979) points out that the outcome depends upon the different combinations of species, site, age, stocking, fertilizer type, rate of application, stumpage, rotation, and discount rate. There are many excellent analyses (Miller and Fight 1979, Keipi 1972) but it is unwise to generalize. Nevertheless, a carefully designed fertilization program can pay, and if the possible adverse effects of chemical wastage, stimulation of nitrification, organic matter loss, and watercourse polution are acceptable to the manager, fertilization has much in its favour in some situations.

The costs and benefits of using nitrogen-fixing symbioses is not so clear. In common with many other forest operations it is relatively easy to assess the costs, but difficult to compute the total benefits, especially in dollar terms. The costs and benefits of these symbioses lend themselves to conventional economic analysis. These values are estimated over the rotation and discounted to the present to give an internal rate of return (%) on the investment, in the limited context of costs considered against the value of the additional fibre produced. This tends to underestimate the true value of the symbioses because it is difficult to quantify such bonuses as added soil organic matter, greater ion exchange capacity, increased nitrogen levels, and other factors improving soil fertility.

An example of a cost and benefit analysis of a nitrogen-fixing symbiosis in the limited context of cost vs. value of improved fibre yield is provided by the Atkinson and Hamilton (1978) study of the effects of planting red alder among douglas fir. Douglas fir seedlings were planted in 1928 in southeast Washington, USA, at 680 trees $acre^{-1}$ (1 680 trees ha^{-1}). Some plots were later (1932) planted with 1 210 red alder per $acre^{-1}$ (2 989 ha^{-1}), and other areas were left as pure douglas fir. The stands were examined in 1975 and it was found that the pure stands had 2 323 ft^3acre^{-1} (160 m^3ha^{-1}) compared with 2 700 ft^3acre^{-1} (190 m^3ha^{-1}) of douglas fir plus 1 940 ft^3acre^{-1} (140 m^3ha^{-1}) of alder totalling 4 641 ft^3acre^{-1} (320 m^3ha^{-1}) in the mixed plots. The average size of the douglas firs in the mixed plots was 14.1 ft^3 (0.4 m^3) compared with 7.7 ft^3 (0.2 m^3) in the pure douglas fir plots. The alder obviously greatly increased tree size and yield per unit area.

A parallel experiment was carried out using nitrogen fertilizers in a pure stand of douglas fir. The fertilization resulted in a yield of 3 100 ft^3acre^{-1} (220 m^3ha^{-1}), i.e. less than in the alder-douglas fir plots, but more than in the untreated pure plots. The average tree contained 10.3 ft^3 (0.3 m^3) of stemwood. Although alder gave a greater yield, the cost of planting 1 210 alder on each acre (2 989 alder ha^{-1}) was high, and consequently the fertilizer treatment was slightly more cost effective than the alder treatment. Nevertheless, the use of 1 210 alders $acre^{-1}$ (2 989 alder ha^{-1}) is probably excessive and the use of fewer alders would make the alder treatment economical if fewer alders produced enough nitrogen. On the basis of investigations on the same site, Miller and Murray (1978) concluded that 20 to 40 uniformly distributed red alder on each acre (49 to 98

alder ha^{-1}) were adequate. These authors concluded in a later paper (Miller and Murray 1979) that using red alder in some douglas fir systems is silviculturally and economically feasible.

Beuter (1979) reviewed the future price of fertilizers, the available evidence on the effects of nitrogen-fixing symbioses, and the economics of using alders, in the context of the entropy law. He concluded that widespread, long-term gains are more likely to come from enhancing nitrogen fixation than from artificial fertilization.

The disadvantage of using nitrogen-fixing symbioses in silviculture is that they introduce complexity into the system and require skill to manage and control. The simplest situation (which parallels agriculture) to manage is the rotation of legumes or actinorhizal plants with mini-rotation (less than 5 years) poplars, willows (Salix spp.), or sycamores. The trees can be cut, symbiotic plants established, and the latter subsequently removed either by closing the tree canopy or by herbicides. In the true forest situation management of the symbioses is more complex.

On the other hand the use of the nitrogen-fixing symbioses is a benign technique of long duration, it uses solar energy rather than non-renewable fossil fuels, and provides many bonuses such as providing organic matter and possibly fibre. The forester will need to decide which soil improvement technique to use taking into account all these factors.

10.0 CONCLUSIONS

i The intensification of forest mangement and harvesting will increase pressures on relatively infertile forest soils and, in some cases, reduce levels of soil nitrogen and organic matter. Foresters will have to manage soils better, as well as trees, if site productivity is to be maintained.

ii While nitrogen fertilizers are easy to apply and often give significant increases in yield in Canada, they do not resolve the problem of organic matter loss and may even accelerate the latter by stimulating nitrification.

iii In the short term, costs of nitrogen fertilizer are not expected to increase faster than the rate of inflation because of abundant supplies of natural gas, the main feedstock. However, the manufacture of nitrogen fertilizer consumes large amounts of energy and natural gas which is a non-renewable resource. In the longer term, fertilizer prices are likely to increase considerably.

iv It is highly desirable that benign, cost-effective, biological soil amendment techniques are developed to supplement forest fertilization. This is particularly important for nitrogen, because nitrogen fertilizers are expensive and can have undesirable effects on the soil microorganisms concerned with mineralization and nitrification.

v Nitrogen-fixing symbioses of *Rhizobium* with legumes and *Frankia* with non-legumes are potentially valuable forest soil amendment tools. They can fix up to 300 kg ha^{-1} $year^{-1}$ of nitrogen in temperate climates, more than enough to replace nitrogen loses by intensive management and harvesting. They are also capable of increasing soil organic matter, and appear to reduce the risk of infection of accompanying trees from some root pathogens.

vi Free-living, nitrogen-fixing soil organisms generally contribute relatively little (less than 5 kg ha^{-1} $year^{-1}$) combined nitrogen to forest sites. Nevertheless, some unexplained high levels of non-symbiotic nitrogen fixation in forest soils suggest that possibly the free-living and associative bacteria in rhizospheres of forest plants may be contributing more combined nitrogen to forest soils than is presently realized. This possibility needs examination.

vii Although legumes can be used on sites where the tree canopy is either open or removed, and could be of great value in mini-rotation poplar systems, the actinorhizal non-legumes such as *Alnus*, *Comptonia* and *Myrica* have the greatest promise in Canadian forestry. The alders have the advantage in that they provide wood fibre.

viii Alders in Canada include the fast-growing *Alnus rubra*, and the shrubby *Alnus crispa* and *A. rugosa*. The Eurasian *Alnus incana* and *A. glutinosa* also grow well in some parts of Canada and many Asian

species remain untested. Very little is known about the highly variable alder populations of Canada and this vast pool of genetic material requires analysis.

ix There are major opportunities for the genetic selection and improvement of alder in Canada. Alders are known to hybridize, and it may be possible to combine the good stem forms of A. rubra, A. incana, and A. glutinosa with the hardy shrubby A. rugosa and A. crispa. There are also opportunities to exploit the polyploidy that is a feature of the genus Alnus.

x There are many examples in the literature of alders and other actinorhizal non-legumes improving levels of nitrogen and organic matter in forest soils, and increasing the growth of associated tree species (by up to 70%). Nevertheless, there is an urgent need to establish well-designed field experiments using plants with nitrogen-fixing symbioses on a variety of sites to demonstrate the impacts.

xi There is a need to develop silvicultural systems that permit the use of rhizobial and actinorhizal symbioses, taking into account the autecology of both the hosts and the microbial symbionts.

xii Although much is known about the ecology of Rhizobium and its legume hosts, very little is known about the ecology of Frankia and its non-legume hosts. This gap in our knowledge needs filling. The techniques for handling and inoculating with bacteria already exist.

xiii Alders have much to recommend them. They produce useful timber (e.g. red alder), and are attacked by pests which, except for the introduced sawflies, are not too difficult to control biologically or chemically.

xiv The use of alders, which contribute fibre and improve the soil, can be economic compared with fertilization, but the high cost of planting alder makes it necessary to use the minimum number of these trees that will have the desired effect. This problem of the optimum number of nitrogen-fixing plants per unit requires resolution.

xv Nitrogen fixation by diazotropic bacteria occurs in the context of the populations of other soil organisms, and it is necessary to understand these interactions. The role of mycorrhizae in supplying the symbioses with phosphorus and possibly providing a pathway for fixed nitrogen from the symbioses to other plants in the ecosystem needs examination.

xvi Nitrogen-fixing symbioses are best considered as another tool for forest management, as a supplement to fertilization rather than a substitute. If the symbioses are used the forester will need to modify his fertilization technique to provide elements for the main tree crop, the host plant, and the nitrogen-fixing processes of the

bacterial symbionts. Elements such as molybdenum, magnesium, and phosphorus are necessary for the nitrogenase enzyme to combine nitrogen and hydrogen, and may necessitate application when levels in the forest soil are too low.

xvii Nitrogen-fixation occurs in many niches in forest ecosystems including the soil, the rhizosphere, plant roots, lichens, tree leaves, rotting logs, insect guts, and mammal rumens. Their full contribution to the nitrogen budget is not understood, and requires investigation.

xviii Nitrogen-fixing symbioses can, therefore, contribute significantly to forestry by reducing the need for costly, energy-demanding nitrogen fertilizers and improving soil organic matter. They deserve serious attention.

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